DISSERTATION ON

CD10- A NEW PROGNOSTIC STROMAL MARKER IN BREAST CARCINOMA, IT'S UTILITY, LIMITATIONS AND ROLE IN BREAST CANCER PATHOGENESIS

Dissertation submitted to
TAMIL NADU DR. M.G.R.MEDICAL UNIVERSITY
CHENNAI

for
M.D. (PATHOLOGY)
APRIL 2016
Under the guidance o f
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CERTIFICATE

This is to certify that this dissertation titled CD10- A NEW PROGNOSTIC STROMAL MARKER IN BREAST CARCINOMA, IT'S UTILITY, LIMITATIONS AND ROLE IN BREAST CANCER PATHOGENESIS is the original and bonafide work done by **Dr.C.ARUN PRABHAKARAN** under the guidance of Dr.P.Arunalatha,M.D., Professor, Department of Pathology at the Government Stanley Medical College & Hospital, Chennai – 600 001, during the tenure of his course in M.D. Pathology from July-2013 to April- 2016 held under the regulation of the Tamilnadu Dr. M.G.R.Medical University, Guindy, Chennai – 600 032.

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DECLARATION BY THE CANDIDATE

I solemnly declare that this dissertation titled "CD10- A NEW PROGNOSTIC STROMAL MARKER IN BREAST CARCINOMA, IT'S UTILITY, LIMITATIONS AND ROLE IN BREAST CANCER PATHOGENESIS**"** is the original and bonafide work done by me under the guidance of Dr. P.ARUNALATHA, M.D., Professor, Department of Pathology at the Government Stanley Medical College& Hospital, Chennai – 600 001, during the tenure of my course in M.D. Pathology from AUGUST -2013 to April-2016 held under the regulation of the Tamil Nadu Dr. M.G.R.Medical University, Guindy, Chennai– 600 032.

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ABBREVIATIONS

BIRADS	:	Breast imaging reporting and data system
BRCA1	:	Breast cancer1
CD10	:	Cluster of differentiation
DAB	:	Diaminobenzidine
DCIS	:	Ductal carcinoma in situ
DPX	:	Distyrene, Plasticizer (tricresyl phosphate), xylene
ER	:	Estrogen receptor
FSH	:	Follicle stimulating hormone
GnRH	:	Gonadotropin releasing hormone
HER2neu	:	Human epidermal growth factor receptor 2
HPF	:	High power field
HRP	:	Horse radish peroxidase
IHC	:	Immunohistochemistry
LH	:	Luteinizing hormone
MiB1	:	Mindbomb 1
PIP3	:	Phosphatidylinositol trisphosphate
PTEN	:	Phosphatase andtensin homolog
PR	:	Progesterone receptor
SHBG	:	Sex hormone binding globulin
TNM	:	Tumor node metastasis
TRIS	:	tris-(hydroxymethyl)aminomethane

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INSTITUTIONAL ETHICAL COMMITTEE,

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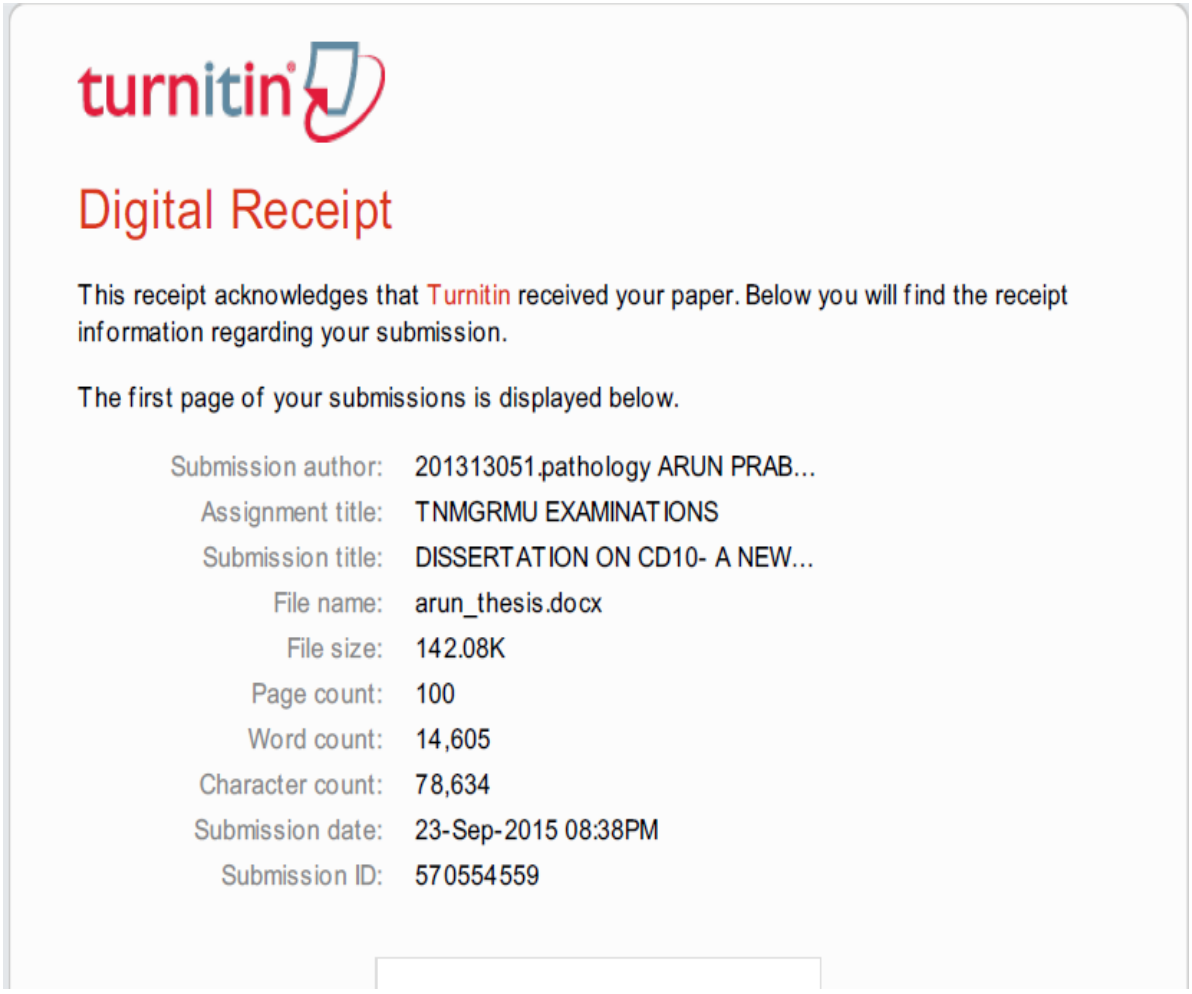

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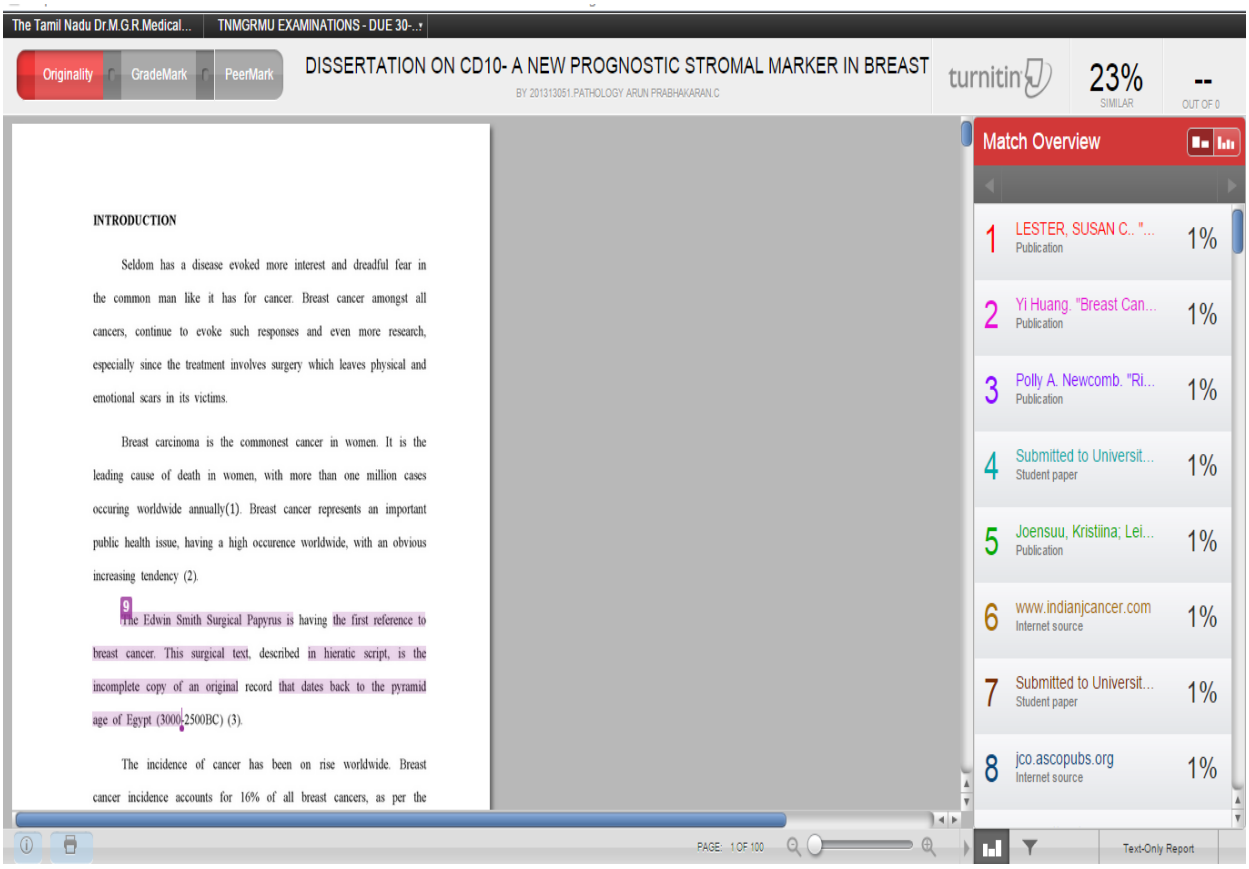
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INTRODUCTION
Seldom has a disease evoked more interest and dreadful fear in the common man like it has for cancer. Breast cancer amongst all cancers, continue to evoke such responses and even more research, especially since the treatment involves surgery which leaves physical and emotional scars in its victims.
Breast carcinoma is the commonest cancer in women. It is the leading cause of death in women, with more than one million cases occuring worldwide annually(1). Breast cancer represents an important public health issue, having a high occurence worldwide, with an obvious increasing tendency (2).
The Edwin Smith Surgical Papyrus is having the first reference to breast cancer. This surgical text, described in hieratic script, is the incomplete copy of an original record that dates back to the pyramid age of Egypt (3000-2500BC) (3).
The incidence of cancer has been on rise worldwide. Breast cancer incidence accounts for 16% of all breast cancers, as per the
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The incidence of cancer has been on rise worldwide. Breast cancer incidence accounts for 16% of all breast cancers, as per the WHO cancer control and prevention program. It is calculated that 519 women died owing to breast malignancy in 2004 (4). Inspite of the fact, breast cancer is thought to be a disease of the developed world, majority of breast cancer mortality (69%), is in developing countries (4). Hence

breast cancer has emerged to be one of the leading cancer killers amongst women worldwide.

Over the last few decades there have been better advances in breast cancer. Early detection and skillfull treatment has lead to a significant decline in breast cancer deaths. It has also made improved outcome for women living with the disease. Breast cancer is no longer seen as single disease but rather a multifaceted disease consisting of diverse biological subtypes with distinct natural history. Breast cancer presents as a varied spectrum of clinical, pathological and molecular features with diverse prognostic and therapeutic implications.

Estrogen is the steroid hormone, responsible for development and maturation of primary and secondary sexual characteristics in females (5). Estrogen has an important role in pathogenesis and development of breast cancer (6).

Estrogen receptor is an intracellular protein molecule. They are targets for estrogen action. Estrogen receptor normally resides in cell nucleus, along with DNA molecules. Estrogen receptor alpha gene polymorphism leads to alteration in estrogen receptor function in breast cancer (7).

AIMS AND OBJECTIVES

- To study the stromal expression of CD10 in breast carcinoma
- To study relationship with certain prognostic factors like,
    1. Age,
    2. Nottingham's grade
    3. ER, PR, HER2neu
- To study the role of stroma in the pathogenesis of breast cancer
- To study the role of CD10 in triple negative cases

REVIEW OF LITERATURE

Breast anatomy and development provide a foundation for understanding the types of breast cancer that occur and the hormonal factors that influence breast cancer cell growth.

Normal breast tissue:

Human breasts are composed of parenchymal tissues consisting of a branching ductal system radiating from the nipple. The breast parenchyma consists of 15-20 mammary lobes. Each lobule drains to nipple by lactiferous duct. They are seperated from one another by interlobar connective tissue. Just before entering the nipple, each of the 15-20 main ducts expands into a dilated segment called the lactiferous sinus. Each lobe consists of 30-80 lobules, which contain the milk-producing elements of the breast. The lobules in turn are composed of 20-40 terminal units or acini, which are surrounded by hormonally responsive intralobar connective tissue (8). The proportion of fat, fibrous and parenchymal tissue vary greatly between individuals and with menopausal status, weight, number of live births and genetic factors (9).

Rudimentary breast development begins in utero and then the anatomy undergoes distinct changes at the puberty, during menstruation, with pregnancy and lactation and finally at menopause. At birth, a female infant has nipples and rudimentary ductal system. At puberty,

under the influence of GnRH, anterior pituitary secretes Follicle stimulating hormone(FSH) and Lutenizing hormone (LH) . FSH and LH in turn stimulates ovaries to produce estradiol .

The Estrogens, primarily 17-estradiol, stimulate the growth and development of breasts. It takes one to two years after menarche before the ovarian follicles are fully mature and begin to ovulate and produce progesterone. Estrogen and progesterone together contribute to the full development of ducts, lobules and alveoli. Fluctuations in estrogen and progesterone levels during a normal menstrual cycle influences breast morphology. During the first half of the menstrual cycle (follicular phase), under the influence of FSH and LH, estrogen levels increase and peak halfway through the cycle. Ovulation occurs and then a second peak of estrogen occurs in the second half of the cycle (luteal phase) when the progesterone levels peak. Estrogen and progesterone promotes the development of ducts and alveoli respectively in the mammary glands (11).

CARCINOMA OF THE BREAST

Breast cancer develops due to uncontrolled growth of the epithelial cells at the junction of the terminal duct-lobular unit. It has been calculated that most breast cancers need about 5-10 years to develop from a single malignant cell to a tumor of 5-10mm diameter (12).

EPIDEMIOLOGY

Globally, breast cancer is the most common neoplasm affecting females. It comprises about 25% of all new cases of cancer (13). Breast cancer incidence is low in less developed countries and in Japan than in industrialized countries. In 2000, global incidence of breast cancer was over 10 million in which 5.5 million cases were seen in developing countries (14).

Cervical cancer incidence is higher than breast cancer in **developing countries**, the reverse holds true in **developed countries**.

In India, breast cancer is the **second most common** cancer in women after cervical cancer. However in Indian metropolitan areas, breast cancer has become the most common cancer than cervical cancer (15). Incidence of breast cancer is 21% for the year 2015.

Breast cancer incidence rate shows **geographical variation** largely because of **socio-economic, reproductive, hormonal, nutritional and genetic factors**. Highest incidence rates are seen in North America and

Europe. Asia and Africa has the lowest incidence rates of breast cancer (16).

After remaining constant for many years, the incidence of breast cancer has begun to increase. This is due to detection of increased number of cases by means of introduction of **mammographic screening** in early 1980's (17).

The main aim of screening is the detection of in situ carcinomas small predominantly ER positive invasive carcinomas. DCIS is almost exclusively detected by mammography, providing an explanation for increase in the diagnosis of DCIS since 1980 (17). In the age of screening, the number of stage I cancers (small node negative carcinomas) has increased in frequency, while the number of large node positive or advanced stage breast carcinomas has fallen (17).

During the 1980's there was increase in incidence of breast cancer but the number of deaths remained constant. Since 1994, the breast cancer mortality rate for all women has slowly declined from 30% to 20%. The **decrease** is **attributed** to the detection of clinically significant cancers at a **curable stage due to screening**, as well as **better and effective treatment modalities** (18)

From 2001 to 2004, the incidence of Estrogen receptor positive invasive breast cancer has raised. The reason for this trend is multi

factorial. This may be attributed to the fact that, in 2002 many women stopped using hormone replacement therapy.

ETIOLOGY AND RISK FACTORS

Breast cancer is a multi factorial disease, since a variety of factors contribute to the biologic processes involved in carcinogenesis. Some of these factors are genetic changes in oncogenesis and in tumor suppressor genes, growth factor imbalances, enzyme production and telomerase activity. These genetic changes interact with non genetic factors such as environment, nutrition and other lifestyle risk factors leading to cancer. By identification of modifiable risk factors and controlling them, the risk of breast cancer has been lowered.

Any factor, such as **ovarian hormones** and **growth factors** that increases cellular proliferation in breast epithelium raises the risk. Increased cell proliferation increases the opportunities for spontaneous genetic damage leading to breast cancer risk (19).

I.Reproductive risk factors:

A. Early menarche and late menopause:

Early age at menarche increases risk of breast cancer. In general, for every one year delay, breast cancer frequency decreases by 10-20%. Both age of onset of menarche and regular cycles influence the risk of

breast cancer. Breast cancer risk may be explained by effect of early menarche on estrogen level (20)

Women with surgically induced menopause have been shown to have reduced risks of breast cancers compared to women whose menopause occured naturally. In comparison with women whose menopause occurs between the ages of 45 and 54(relative risk 1), women with late menopause at age more than 55 years have a relative risk of 1.48. Increased risk of breast cancer in late menopause is due to long menstrual history and ovarian function (21).

B. Parity

Early age at first pregnancy decreases risk of breast cancer.

17-41% reduction in breast cancer risk is seen in parous women when compared to nullipara (22). This reduction is not immediately found in parous women. Actually the risk is increased in the first ten or more years following pregnancy. It may be explained by proliferative changes in pregnancy. Breast cancer risk is decreased by 7% for every child birth thereafter. This reduction is attributed to maximum differentiation in breast parenchyma in which further DNA damage does not take place.

C. Age at first live birth

Early first pregnancy leads to maturation of terminal ductal lobular unit of breast thereby reducing risk of breast cancer. Hence, women who

are more than 35 years of age have 60% increased risk in breast cancer than those who are less than 18 years of age at first pregnancy (23).

D. Breast feeding

Breast feeding further reduces the risk of breast cancer in parous women. There is about 12% decrease in relative risk of breast cancer development for women who breast feed for one year (24). This reduction percentage is increased upto 50% in high parity females.

Breastfeeding is thought to decrease breast cancer risk by lessening the total number of menstrual cycles and consequently cumulative ovarian hormone exposure.

E. Hormones

Reproductive risk factors are well known to influence breast cancer risk by modulating endogenous hormone levels. In the following sections, the relation between serum hormonal levels and breast cancer risk will be discussed.

F. Estrogens and Androgens

Estrogen increases cell proliferation in the breast. In premenopausal women, nearly all estrogen is of ovarian origin. After menopause, direct ovarian production stops and most estrogen is derived from the aromatization of adrenal androgens.

Estradiol and estrone sulfate are the types of estrogen implicated in breast cancer development. The 17β estradiol is the most functionally active form of estrogen from puberty till menopause. Estradiol circulates in the blood either as free hormone or bound to sex hormone –binding globulin (SHBG) and albumin. Free estradiol or estrogen, which binds to albumin are functionally more active forms. Major circulating estrogen is estrone sulfate. They are the major resource of estrogen from adipose tissue in postmenopausal females.

Androgens such as testosterone and androstenedione can be aromatized into estrogens, either in the ovaries or in adipose tissues. Estrogen is derived directly from ovary and adrenal gland as well as from the peripheral conversion of androstenedione.

Breast cancer risk is **directly proportional** to the levels of serum **concentrations of sex hormones** including total and free estradiol, androstenedione and testosterone (25).

Serum estradiol levels have been shown to be be **less in Asian women** regardless of menopausal status. These differences seen in low risk population may be due to reduced number of ovulatory cycles as a result of late age at menarche, higher parity, frequent breast feeding, breast feeding for longer durations, and early age at menopause.

In postmenopausal women, **weight is directly proportional** to plasma levels of estrone and estradiol, as well as unbound estradiol to

SHBG. Hence **postmenopausal obese women** have **greater risk** of breast cancer development than in non obese women.

G. Hormone replacement therapy

Invasive breast cancer occur more among the current users of hormone replacement therapy especially those who have used more than five or more years. However the risk of breast cancer is no higher among former users who have stopped taking hormones more than 5 years previously, than the risk among never users. Hence the major consequence of hormone replacement therapy is promotion of cancer growth rather than direct genotoxic effect.

Breast cancer risk is increased by 2.3% for each year among women using hormone replacement therapy currently, or who have stopped within one to four years. The relative risk for breast cancer is 1.35 for women who had **used hormone replacement therapy for more than 5 years**.(26)

II. Anthropometric risk factors

The correlation between weight and breast cancer risk differs according to menopausal status. Increased weight or BMI has been shown to lessen breast cancer development in premenopausal but increases risk in post menopausal women.

Several hypothetized mechanisms exist to explain the low risk of breast cancer in obese premenopausal women. Obese premenopausal women have decreased progesterone levels because obesity may cause anovulation and a reduced progesterone secretion in the luteal phase. Also, leptin levels which increases with increasing fat stores, inhibit ovarian estrogen production, and may thereby decrease breast cancer development in obese premenopausal women.

Obesity increases breast cancer risk in postmenopausal women by increasing levels of endogenous estrogen (27). The principal source of estrogen in postmenopausal female is the conversion of androstenodione to estrone in adipose tissue. Also, sex-hormone-binding globulin levels fall when BMI is increased, thus increasing the levels of free estradiol. In addition, obesity may increase the concentration of several circulating cytokines, which stimulate the activities of the enzymes, involved in the synthesis of estrogen.

III. Family history of breast cancer / Genetic factors

Family history of breast cancer is one of the most well-established risk factor for breast cancer. Some family history is important, while others are of little consequence. Most women who have relatives, who have developed breast cancer post menopausally are not genetically predisposed to breast cancer and their increased risk is low. On the other hand, the women who have first degree relatives with breast

cancer have substantially increased risk of breast cancer. Risk is about 1.5-2 times above in the woman who has no affected first degree relative. The risk may be further increased to 6 if more than one first degree relative has been affected. Cancers develop in this population at an earlier age in their mother or sister. Also they have inherited DNA mutation of BRCA 1 or BRCA 2 gene that increases the risk of breast cancer.

Lynch distinguishes familial breast cancer from hereditary breast cancer. Familial breast cancer is defined as "Family having more than two first degree relatives with breast cancer in the absence of hereditary breast cancer". Hereditary breast cancer is defined as "Pattern within a particular family having Mendelian segregation of breast cancer". The former are probably events that may happen, by the laws of probability to cluster in a family, while the latter cancers are likely the results of inheritance of abnormal DNA (28)

Genetic factors have a role in approximately 5% of all breast cancer cases. But the risk percentage increases to 25% in cases below 30 years of age. Several genes are implicated in breast cancer development. BRCA 1 gene located on chromosome 17 and BRCA 2 present on chromosome 13 are associated with majority of inherited breast cancers. 2-5% of breast cancers are hereditary. BRCA 1 and BRCA 2 are the tumor suppressor genes with numerous important cell

functions. It includes gene transcription, regulation of cell cycle check points and DNA repair.

Many genes other than BRCA are involved in breast cancer risk. Women with Li-Fraumeni syndrome have increased risk in development of early onset of many cancers including breast cancer. This syndrome is due to mutations in p53 tumor suppressor gene. In Ataxia telangiectasia, there is 100 fold increase in breast cancer risk in women. It is an autosomal recessive syndrome due to DNA repair defect. Women with Cowden disease having mutation in the PTEN tumor suppressor gene develop breast cancer by 50 years of age.

Hence, breast cancer may also develop due to alleles with low to moderate penetrance. They confer lesser risk, but the attributable risk is more when it is common in the population. Breast Cancer Association study implies that larger sample size is required to clarify association of polymorphism with breast cancer (29)

Genetic susceptibility due to both high and low penetrance gene mutation, together with interaction of environmental factors leads to increased incidence of breast cancer.

IV. Other risk factors

A. Benign breast diseases

Certain types of benign breast diseases have increased risk of breast cancer. There is 1.5 fold increased risk of breast cancer for those women with benign breast disease without hyperplasia compared to normal population. The risk of breast cancer among women with hyperplasia varies with whether atypia is present or not. Atypical hyperplasia increases 2.6 fold risk of breast cancer as compared to 1.8 fold increased risk in hyperplasia without atypia.

The breast cancer risk associated with benign breast disease differs by menopausal status. Atypia in premenopausal women have higher relative risk of breast cancer than in post menopausal women (30)

B. Mammographic density

Mammographic density is a strong risk factor for breast cancer. It represents connective and epithelial tissues in the breast, whereas the dark radiolucent areas on the mammogram are primarily fat. Women with highest mammographic density are 4-6 times more likely to develop breast cancer than very low density (31)

C. Ionizing radiation

Ionizing radiation has increased risk of developing breast cancer. Information on radiation and breast cancer risks has come mainly from

epidemiological studies of atomic bomb survivors or women exposed to radiation for diagnostic or therapeutic reasons. Relative risks vary from 1.2 -2.4 and are related to both total dose and age at exposure. Younger women had greater risk than older women (32). The effect of very low doses such as those incurred in occupational exposures is uncertain. Because of the low doses involved in screening mammography (200 – 400 mrad) and of the finding that older women were less susceptible to ionizing radiation, the benefit risk ratio for older women would still be larger.

D. Socioeconomic Status

Higher socioeconomic status has a role in breast cancer. Developed countries have much higher breast cancer rates than developing countries. This correlation between breast cancer risk and socioeconomic status has also appeared at both the individual as well as community level. The higher breast cancer risk among well-educated women appears to be attributable to greater exposure to breast cancer risk factors such as later age at first pregnancy, having few or no children, and more frequent use of oral contraceptives and hormone therapy

S.No.	RISK FACTOR	COMPARISON CATEGORY	RISK CATEGORY	RELATIVE RISK
1.	Age at menarche	16 years	Younger than 12 years	1.3
2.	Age at menopause	45 to 54 years	After 55 years	1.5
3.	Age at when first child born alive	Before 20 years	Nulliparous or older than 30 years	1.9
4.	Benign breast disease	No biopsy or fine needle aspiration	Any benign disease	1.5
			Proliferative disease	2.0
			Atypical hyperplasia	4.0
5.	Family history	No 1st degree relative affected	Mother affected	1.7
			Two first degree relative affected	5.0
6.	Obesity	10th percentile	90th percentile	1.2
7.	Alcohol use	Non drinker	Moderate drinker	1.7
8.	Estrogen replacement therapy	Never used	Current use >3 years	1.5

Table 1 : Risk factors for breast cancer and their relative risks (33)

DIAGNOSTIC MODALITIES

A. Mammography

Screening mammography is used to detect cancer in asymptomatic women. Diagnostic mammography is used to evaluate

1. Patients with breast symptoms or complaints such as nipple discharge or a palpable mass
2. Patient who had abnormal results on screening mammography or
3. Patients who had underwent breast conservation therapy.

The diagnostic examination is tailored to the individual patient specific abnormalities.

B. Digital mammography (Also called full-field digital mammography, or FFDM) –

FFDM is a new technology that was recently approved by the FDA for breast cancer screening and diagnosis**.** They capture the images which are processed on a computer and then viewed.

C. BIRADS diagnostic categories —

After analyzing the mammographic images, radiologists classify findings into a final assessment category. The Breast Image Reporting And Data System (BIRADS), final assessment classification was developed by the American College of Radiology to standardize mammographic reporting. Follow up recommendation are made based on the final assessment category.

D. ULTRASONOGRAPHY

Ultrasound helps to differentiate between solid and cystic breast masses that are mammographically detected or those that are palpated. If there is suspicious metastasis of nodes, ultrasound evaluation of axilla can be done to detect lymph nodes. Interventional procedures can be done for suspicious areas in breast or axilla with ultrasound guidance

E. BREAST MRI

The sensitivity of MRI for breast carcinoma is between 88 and 100 percent. Invasive breast cancer shows contrast enhancement on MRI. Because MRI is so sensitive, it was assumed that preoperative MRI would estimate the extent of disease, more accurately than conventional imaging, thereby improving surgical planning

F. Fine needle aspiration cytology

FNAC of a palpable breast mass can easily proceed in outpatient setting. They are used to differentiate solid and cystic lesions.

The combination of diagnostic mammography, ultrasound and fine needle aspiration cytology achieves almost 100% accuracy in diagnosis of breast cancer

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G. Biopsy

Core Needle biopsy

In core needle biopsy, tissue sample is obtained from the mass by using hollow needle. The advantages of core biopsy are low complication rate, avoidance of scarring and low cost

Open biopsy

An open biopsy is recommended only in patients who have been appropriately investigated by imaging, FNAC, and or by core needle biopsy

H. Morphology

Gross

Classic invasive ductal carcinoma is actually a prototypic expression of breast carcinoma. In typical case, it is firm and poorly circumscribed, with resistant gritty sensation on cutting, and yellowish gray cut surface. Trabeculae radiates through the surrounding parenchyma into the fat, resulting in stab like or stellate configuration (**Figure 1**). Areas of hemorrhage, necrosis and cystic degeneration may be present, if the size of neoplasm is large

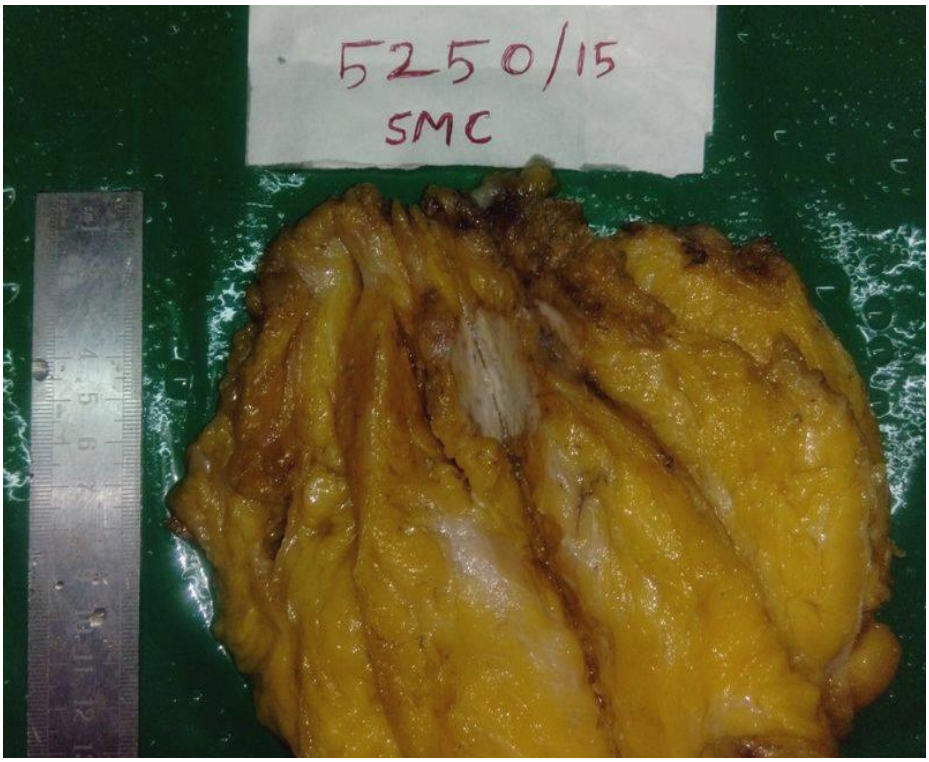


Figure 1: Ill defined grey white growth in central quadrant measuring 3 x 2.5 x 2 cm

Microscopy

The usual growth pattern in microscopy is diffuse sheets, nests, cords or as individual cells. There may be well developed glandular/tubular differentiation or it may be just detectable or it may be completely absent. The tumor cells vary in size and shape, but by definition they are larger and more pleomorphic than those of the classic form of invasive lobular carcinomas, their nuclei and nucleoli are more prominent, and mitotic figures are more numerous. Necrosis can occur in approximately 60% of cases. Foci of squamous metaplasia, apocrine metaplasia, or clear

cell changes may be seen. The amount of stroma may be none or it may be abundant, and its appearance ranges from densely fibrotic to cellular (‘desmoplastic’). If there is abundant stroma, tumor cell identification becomes difficult. Bulky masses of elastic tissue are present in approximately 90% of cases. Sometimes there is chalky streaks on gross examination due to ‘elastosis’ which involves the wall of the ducts and the vessels (mainly veins). Calcification can be detected in approximately 60% of cases, either as coarse or fine granules or, rarely, as psammoma bodies. Chronic inflammatory infiltrate composed of mononuclear cells is usually seen at the interphase between tumor and stroma. Lymph vessel invasion is usually difficult to distinguish from artifactual tissue retraction.

Features used to document the presence of lymphatic tumor emboli are the following: (1) the occurrence of the area in question outside the margin of the carcinoma, (2) the fact that the tumor emboli do not conform exactly to the space in which they lie, (3) the presence of an endothelial cell lining, and (4) the presence of blood vessels in the immediate vicinity.

Immunohistochemically, the tumor cells show reactivity for low molecular weight keratin (particularly types 8, 18, and 19) and EMA. Two other important breast-related markers are

mammaglobin and GCDFP-15, the former being more sensitive but less specific than the latter.

Variants include,

1.Tubular carcinoma

2.Cribriform carcinoma

3.Mucinous carcinoma

4.Medullary carcinoma

5.Invasive papillary carcinoma

6.Invasive micropapillary carcinoma

7.Apocrine carcinoma

8.Secretory carcinoma

9.Carcinoma with neuroendocrine features

10.Metaplastic carcinoma

11.Squamous cell carcinoma

I. Proliferative Biomarkers

High proliferative disease of breast cancer is associated with favourable response to chemotherapy. The main obstacles to use proliferative markers are

1. Poor standardization of detection methods

2. Vaguely defined cutoff values

3. Requirement of fresh frozen tissue

Proliferative biomarkers are

1. **Measurement of cells in S phase:**

Unfavourable prognosis of breast cancer patients are seen when S phase fraction is assessed by fresh or frozen material. But several studies done to assess the prognostic value by DNA flow cytometry lacks standardized procedures, sufficient power and predefined cutoff values. There is also high tumor heterogeneity of the S phase fraction. Therefore it cannot be recommended for routine prognostic assessment. Another disadvantage of this method is requires large quantity of tumor material, making it inappropriate for smaller tumors identified through mammographic screening.

2. **H-Thymidine Labeling Index**

H-thymidine labeling index was one of the proliferative biomarker used in breast cancer. Cells undergoing DNA replication is measured by H-thymidine uptake using autoradiography. Thymidine labeling index represents ratio between the number of labeled and counted cells. A similar approach uses IHC technique and halogenated analogue - Bromodeoxyuridine. Limitations of this technique are the requirement of

fresh frozen tissue, the time required to complete the assay and use of radioactive tracers.

3. **Thymidine Kinase**

Thymidine kinase activity is measured by radio enzymatic assay. Thymidine kinase is an enzyme that catalyses the phosphorylation of deoxythymidine to deoxythymidine monophosphate. Its activity is highest in G1-G translation check point and then reduced in G2 phase of cell cycle. In breast cancer, the fetal isoform of Thymidine kinase is present in high levels in the cytoplasm and regulates the cell cycle.

4. Ki67

Ki67 is a nuclear antigen which is present in mid G1, S, G2 and the entire M Phase of the cell cycle. Over expression of Ki67 correlates with proliferative activity metastases and overall survival.

5. MIB1

Similar to Ki67, MIB1 is a nuclear antigen that can be labeled using immunohistochemistry. It can be performed on formalin fixed and paraffin embedded tissue. A good concordance is seen between Ki67 and MIB1 assessment.

6. Cyclin A

Cyclins are proteins that regulate cell cycle. Cyclin A is expressed mainly in the late S, G2 and M phases of the cell cycle. It has been associated with poor prognosis.

7. Cyclin E

Cyclin E regulates G1 phase progression and entry into S phase. There are two different proteins, cyclin E1 and E2 that are coded by 2 different genes with 47% homology. Cyclin E1 is determined by immunohistochemistry, Western Blot and RT-PCR. Elevated levels of cyclins increases risk of breast cancer related death.

8. Cyclin D1

The family of cyclin D consists of atleast three different cyclins that regulate progression of cell cycle into G1 phase. The function of cyclin D1 is to bind to the cyclin dependent kinases 4 & 6 and phosphorylate downstream proteins. These complexes can sequester cyclin D kinase inhibitors. Cyclin D1 acts as a cofactor for ER alpha in a ligand independent manner. The concentration of Cyclin D1 is highest during mid G1 phase and then gradually declines. Over expression of cyclin D1, mRNA, protein and amplification to account 15% in breast cancer. They are found to be associated with ER positive and well differentiated

tumors. The most common method of detection of cyclin D1 expression is immunohistochemistry.

9. p27

p27 is a cyclin dependent kinase inhibitor that acts in the nucleus. It is mobilized by antiproliferative signals, such as cell to cell contact and transforming growth factor beta. It can be assessed by immunohistochemistry. In majority of the studies p27 was positively correlated with ER expression and had inverse correlation with the grade. In BRCA1/2 mutated tumors, low level of p27 are seen.

10. Topoisomerase II alpha

Topoisomerase II are DNA binding enzymes with nuclease, helicase and ligase activity. Topoisomerase II beta is not cell cycle dependent whereas topoisomerase II alpha is cell cycle dependent and is highest in G2/M transition. Coamplification of topoisomerase II and Her-2 are associated with increased sensitivity to anthracyclines. It is also used as a prognostic marker for overall survival of breast cancer patients independent of therapy

11. **Urokinase plasminogen activator (uPA) and Plasminogen activator inhibitor(PAI-1**)

uPA and PAI-1 levels are associated with breast cancer recurrence and survival. They also predict the hormone therapy and specific types of chemotherapy response.

Staging of breast cancer

TNM staging of tumor is based on size of primary tumor, regional lymph node and distant metastasis.

American Joint Committee on Cancer (AJCC) staging system provides guidelines for breast cancer patient according to the prognostic status. The AJCC has designed staging by TNM classification.(34)

TNM STAGING

Primary tumor (T):

TX: Primary tumor cannot be assessed

T0: No evidence of primary tumor

Tis: Carcinoma in situ; DCIS/LCIS/Pagets

T1: Tumor size (2 cm or less).

T1mi: less than 0.1cm microinvasion

T1a: more than 0.1 cm but less than 0.5 cm

T1b: more than 0.5cm but less than 1 cm

T1c: more than 1cm but less than 2 cm

T2: Tumor size 2-5 cm

T3: Tumor size more than 5cm

T4: Tumor of any size with direct extension to chest wall and or to the skin (ulceration or skin nodules)

T4a: Extension to chest wall, not including only pectoralis muscle invasion/adherence

T4b: Ulceration and/or ipsilateral satellite skin nodules and/or edema

T4c: both of the above (T4a and T4b)

T4d: Inflammatory carcinoma

Regional lymph nodes (N)

NX: (RLN) cannot be assessed

N0: No regional lymph node metastasis

pN0(i-): No 'RLN' metastasis identified histologically, negative IHC

pN0(i+): Malignant cells in 'RLN' less than 0.2 mm (detected by H&E or IHC)

pN0(mol-): No RLN metastasis histologically, negative molecular findings(RT-PCR)

pN0: Positive molecular findings (RT-PCR) but no RLN metastasis detected histologically or by IHC

pN1m1: Micrometastasis (greater than 0.2 mm and /or more than 200 cells but none greater than 2.0mm)

pN1a: Metastases in 1 to 3 axillary lymph nodes, atleast one metastases greater than 2.0 mm

pN1b: Metastases in internal mammary nodes with micrometastases or macrometastases detected by sentinel lymph node biopsy but not detected clinically

pN1c: Metastases in 1 to 3 lymph nodes and in internal mammary nodes with micrometastases or macrometastases detected by sentinel lymph node biopsy but not detected clinically

pN2a: Metastases in 4-9 axillary lymph nodes (atleast one tumor deposit greater than 2.0 mm).

pN2b: Metastases in clinically detected internal mammary nodes and in the absence of axillary LN metastasis

pN3a: Metastases in 10 or more axillary lymph nodes (at least one tumor deposit greater than 2.0mm);or metastases to the infraclavicular (level 3 axillary lymph nodes and in internal mammary lymph nodes) nodes

pN3b: Metastases in clinically detected ipsilateral internal mammary lymph nodes

Distant metastases (M):

M0: No distant metastasis

M1: Distant detectable metastasis as histologically proven larger than 0.2mm

STAGING:

Stage groupings: Stage 0 Tis,N0,M0 Stage 1 T1,N0,M0 T1 include T1mic Stage 2A T0,N1,M0 T1,N1,M0 T2,N0,M0 T1 includes Tmic. The prognosis of patient with pN1a disease is similar to that of pN0 disease Stage 2B T2,N1,M0 T3,N0,M0	Stage 3A T0,N2,M0 T1*,N2,M0 T2,N2,MO T3,N1,M0 T3,N2,M0 *T1 includes T1 mic Stage 3b T4,Any N,M0 Any T,N3,M0 Stage 4 Any T, Any N, M1

Table 2 : Staging of breast cancer

Histological Grading of Breast Cancer

Histologic grading based on

1. Tubule formation, **2**. Nuclear pleomorphism, **3.** Number of mitosis

1.Tubule formation	Score
>75%	1
10 to 75%	2
<10%	3
2. Nuclear pleomorphism	
Small uniform cells	1
Moderate increase in size and variation	2
Marked variation	3
3.Number of Mitosis (Microscope Nikon 40x objective)	
Upto 7/ 10 hpf	1
8-14/ 10 hpf	2
More than or equal to 15/ 10 hpf	3

Table 3 : Modified Bloom Richardson histologic scoring criteria (35)

GRADE	DESCRIPTION	SCORE
Grade 1	Well differentiated breast epithelial cells. Cells generally appear normal not growing rapidly. Cells arranged in small tubules.	3,4,5
Grade 2	Moderately differentiated breast epithelial cells. Have characteristics between Grade 1 & 3 tumors	6,7
Grade 3	Poorly differentiated breast epithelial cells. Cells do not appear normal and tend to grow and spread aggressively.	8,9

Table 4 : Nottingham Modification of Bloom Richardson grading system

STAGE	5 YEAR SURVIVAL RATE
0	100%
I	100%
2A	92%
2B	81%
3A	67%
3B	54%
4	20%

Table 5 : Five year survival rate

Estrogen receptor

The breast cancer markers that are most important in determining therapy are estrogen and progesterone receptor and HER-2/neu

Estrogen receptors are members of the steroid receptor superfamily. Two isoforms of ER such as ER alpha and ER beta have been identified. The knowledge about most of ER activity and function has been obtained from ER alpha studies. Much less is known about ER beta (36).

The ER is a nuclear transcription factor that regulates the expression of number of genes involved in regulation of transcription and differentiation. Binding of ligand to ER results in a ligand-ER complex that subsequently induces an ER conformational change, dissociation of chaperones such as hsp90 and hsp70 and receptor dimerization. Activated ER dimers can bind to the Estrogen receptor elements (ERE) of target genes and regulate their transcription. Some nuclear proteins interact with ER and function as coactivators or corepressors of ER.

Due to the important role played by ER in breast carcinoma progression, development of agents which can be specific to target ER or its ligands is therefore an important strategy for breast carcinoma treatment. Endocrine therapies applied include depletion of the ligand, estrogen, steroidal antiestrogens that destroy ER and selective ER modulators.

Hormone resistance which can be denovo or acquired is a feature of some breast carcinomas. 30% of breast cancers lack ER gene expression approximately. **Tumor lacking ER protein are usually associated with higher growth rate, poor differentiation and worse clinical outcome**. Therefore ER expression can be used as a prognostic factor for early breast carcinoma patients. Genetic changes that account for the loss of ER in breast cancer include deletions, insertions, point mutations or rearrangement of ER gene.

Tissue distribution of ERs:

The distribution of two receptors overlap in breast, endometrium, bone, prostate, epididymis, central and peripheral nervous system (37).

Liver and white adipose tissue show ER alpha expression alone.

Kidney, Bladder, Intestine, ovary show only ER beta expression alone.

Progesterone receptor:

Positive receptor status in breast carcinoma is associated with a good prognosis as well as better response to hormonal therapy, better survival and long disease free period(38). IGF-I (insulinlike growth factor-1) inhibits expression of progesterone receptor in breast carcinoma cells via the phosphatidylinositol 3-kinase/akt/mammalian target of the rapamycin pathway. If there is low expression of PR, it indicates

activated growth factor signaling in breast carcinoma cells (39). Hence low expression of progesterone receptor may serve as an indicator of activated growth factor signaling in breast carcinoma cells, and it represents aggressive tumor phenotype which is resistant to hormonal therapy (39). PR expression can define a subpopulation of breast cancer patients who may be strongly dependent on hormone receptor-associated growth, and so superior response to hormone therapy (40).

PR bind hormones that exert their effects in the nucleus. Nuclear immunostaining for both estrogen and progesterone receptors are normally demonstrated on breast acini, which serve as internal controls for the testing procedure (**Figures 2,3**). In general, approximately 15% to 20% of the luminal epithelial cells in a duct or lobule stain with ER and PR. However, nuclear staining in normal breast tissue may vary with the menstrual cycle and is heterogeneous (41)

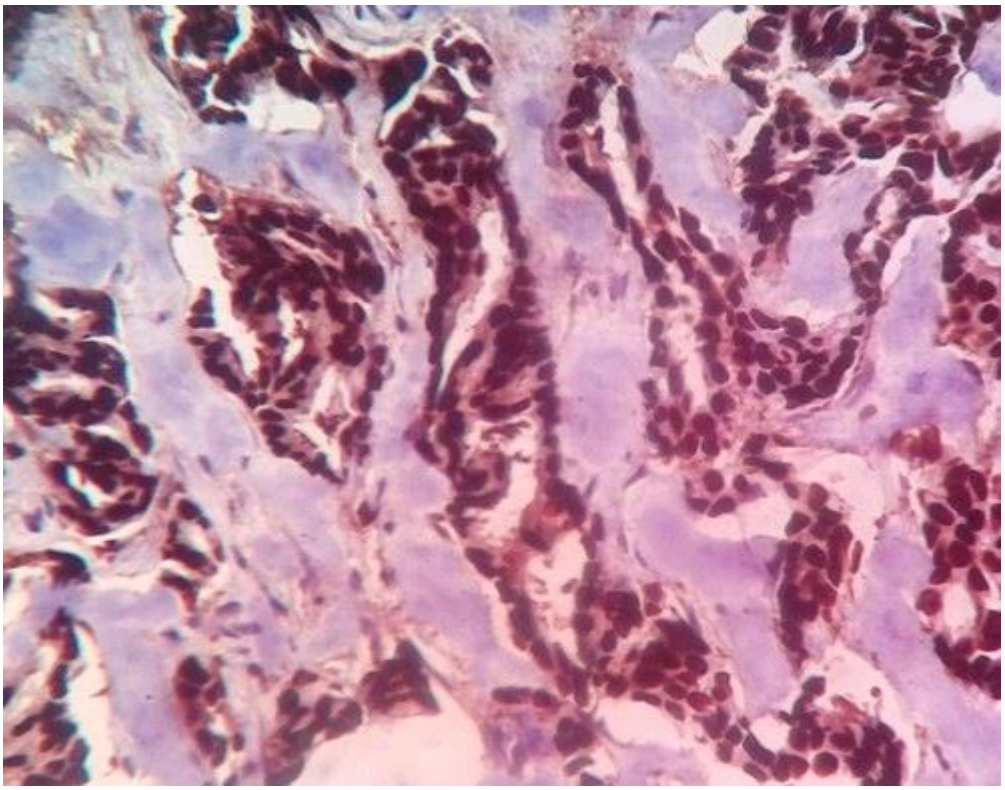


Figure 2 : Estrogen receptor positivity (nuclear staining)

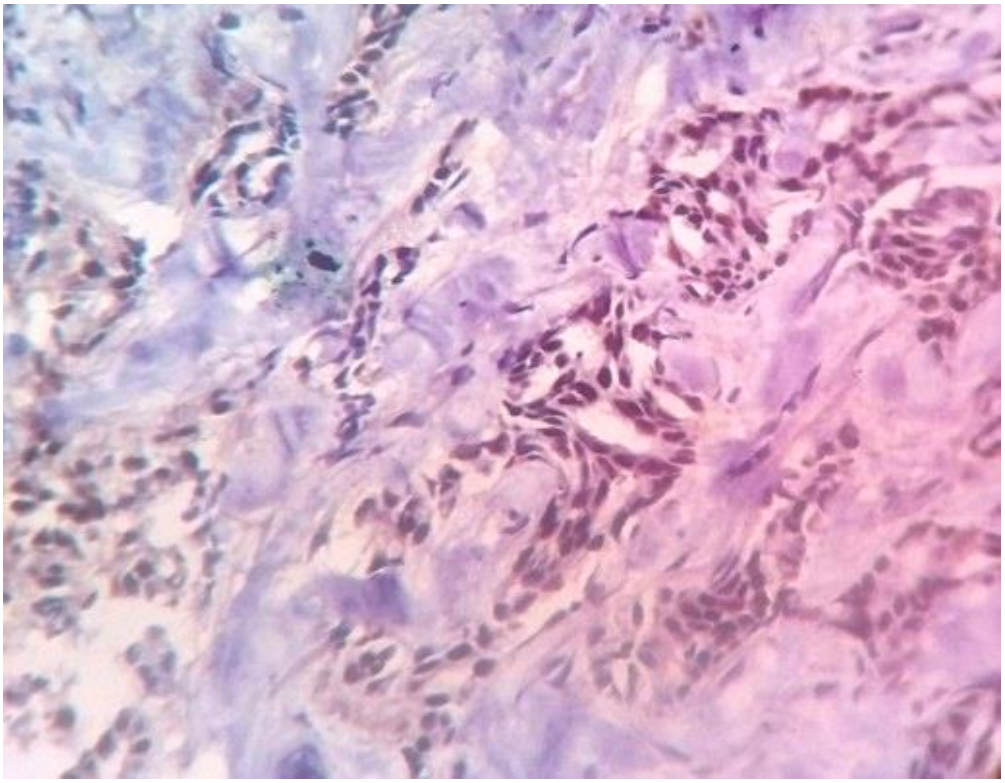


Figure 3 : Progesterone receptor positivity (nuclear staining)

In carcinomas of the breast, most PR-positive tumors are also ER positive, and ER-negative, PR positive tumors account for fewer than 1% of all breast cancers. In general, patients with positive PRs have a significantly longer disease-free survival than patients who are PR negative

HER2neu:

The ERBB2 (formerly HER2) gene was originally called NEU because it was first derived from rat neuroblastoma/ glioblastoma cell lines. Coussens and colleagues (42) named it HER2 because its primary sequence was very similar to human epidermal growth factor receptor (EGFR, ERBB, or ERBB1). Semba and associates (43) independently identified an ERBB-related but distinct gene, which they named ERBB2. Di Fiore and colleagues (44) indicated that both NEU and HER2 were the same as ERBB2, and Akiyama and associates (45) precipitated the ERBB2 gene product from adenocarcinoma cells and demonstrated it to be a 185-kD glycoprotein with tyrosine kinase activity

Clinical significance of HER2 gene amplification was shown in breast cancer, in the year 1987 (2 years after its discovery) (46). 15% to 20% of breast cancers approximately demonstrate HER2 gene amplification and/or protein overexpression (47,48). HER2-positive breast cancer patients have a worse prognosis without any adjuvant systemic therapy. They have higher rates of recurrence and mortality, so it clearly demonstrates significant prognosis.

An even more important aspect of determining HER2 status is its role as a predictive factor. HER2 positivity is predictive of response to anthracycline- and taxane-based therapies, although the benefits derived from non-anthracyclines and non-taxane therapies may be inferior (49-53). It is also important to note that HER2- positive tumors generally show relative resistance to all endocrine therapies; however, this effect may be more toward selective endocrine receptor modulators, such as tamoxifen, and less likely toward estrogen-depletion agents, such as aromatase inhibitors (54,55).

HER2neu takes up membranous pattern of staining (**Figure 4**)

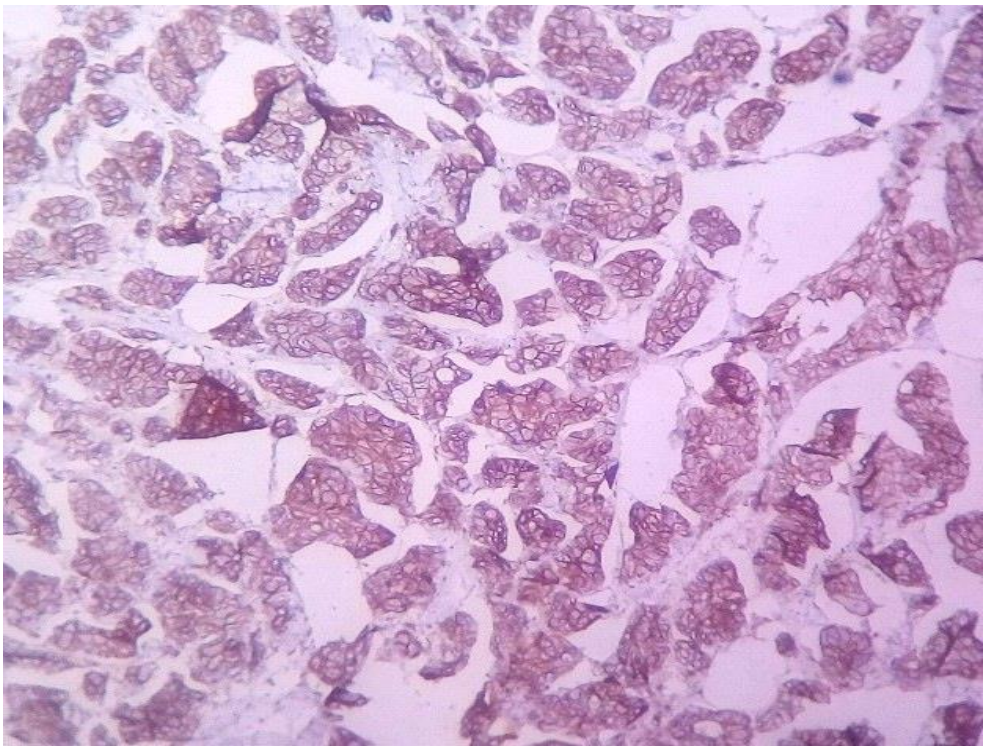


Figure 4 : HER2neu positivity (membranous staining)

CD10:

It is a 90-110 kDa cell matrix metalloproteinase, which is a membrane bound zinc dependent endopeptidase. It is also called as "common acute lymphoblastic leukemia antigen" (58), "neutral metalloendopeptidase" in kidney and "enkephalinase" in brain (62). Matrix metalloproteinases are metallopeptidases which cleaves extracellular matrix proteins and play an important role in tissue remodeling. In normal breast, it lowers the extracellular concentration of many peptides available for receptor binding and thereby regulates their physiological action. It cleaves signaling proteins that usually promotes differentiation of early common progenitors to luminal epithelial progenitor or myoepithelial progenitor, which gives rise to luminal and myoepithelial cells and thereby maintains the early progenitor population. So it acts as a stem cell because of its enzymatic function and prevents uncontrolled proliferation (56).

In carcinoma, absence of CD10 expression from myoepithelial cells leads to progression from DCIS to invasive carcinoma and its stromal expression correlates with poor prognosis, higher grade and negativity of estrogen receptor. Early oncogenic events in stem cells modulate CD10 expression in altered cells, hence it results in reduced enzymatic function of CD10 which leads to accumulation of unprocessed peptides and finally malignant transformation. But the enzymatic activity

of CD10 can be upregulated in invasive carcinoma, mostly in mesenchymal stem cells or from proliferation of transformed epithelial cancer stem cells. So this results in accumulation of local CD10 cleaved peptides which maintains cancer stem cells and inhibits epithelial cell differentiation (56).

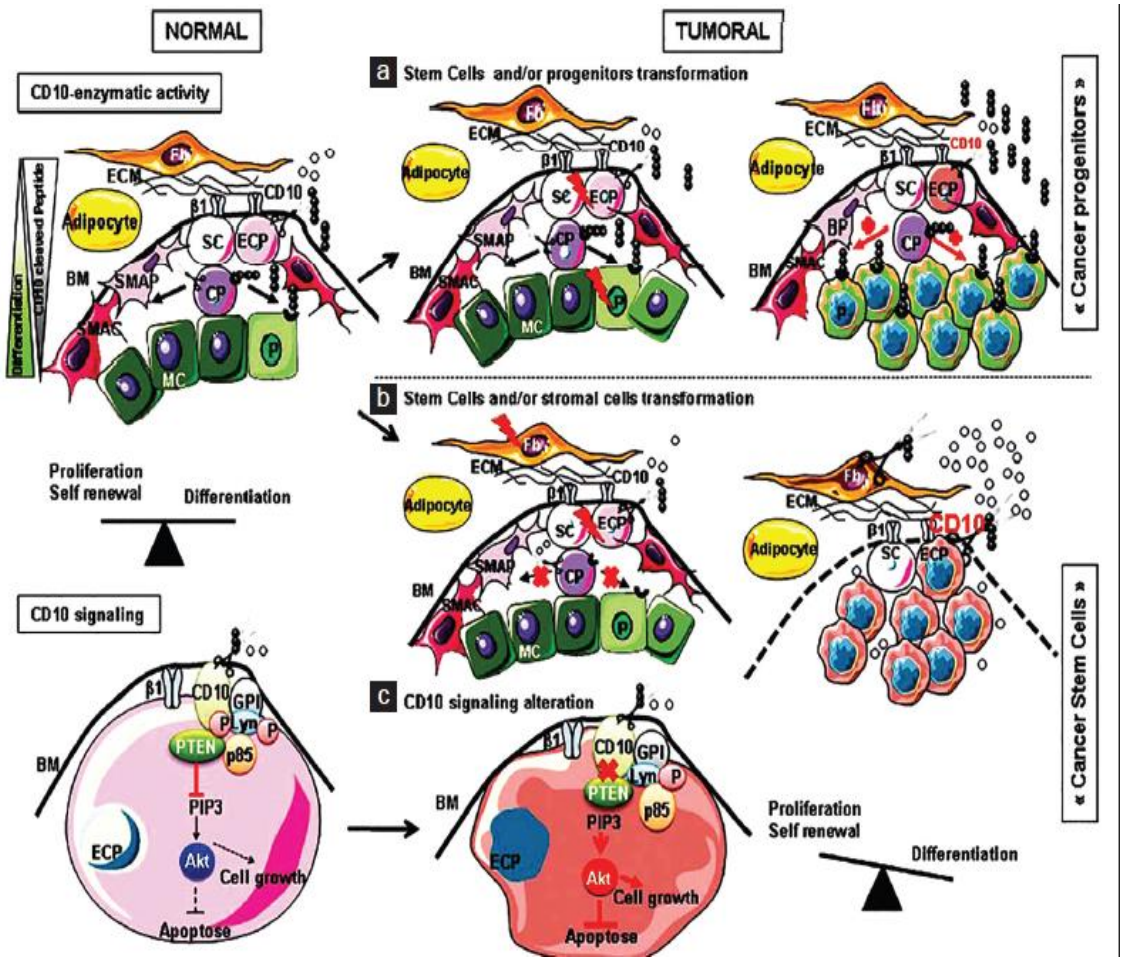


Figure 5 : CD10 pathway

CD10 in normal tissue associates with tumor suppressor PTEN leading to decreased phosphorylation of PIP3, which prevents Akt

pathway activation and leads to cell apoptosis. CD10 cleaves growth factors like fibroblast growth factor 2 (FGF2), which induces Akt signaling in favor of endothelial cell growth and angiogenesis. CD10 prevents cancer cell migration. Signaling of CD10 is modified in cancer progenitors or stem cells which blocks PTEN function. It leads to inhibition of apoptosis, increased proliferation of cells and angiogenesis. According to molecular studies, CD10 positivity is correlated with ER negativity and HER2 positivity. Basal like (ER-/PR-/Her2-) and HER2E(ER+/HER2+) have strong association with PTEN loss than luminal type (ER+) (56). CD10 staining can be observed in both stroma and extracellular matrix because neutral endopeptidase is produced by specialized tumor stromal cells and after secretion, is involved in degradation of extracellular matrix. Gene expression profiling study of stroma in breast carcinoma has identified two significant types of stromal signature, namely solitary fibrous tumor type and desmoid-type fibromatosis type. CD10 expression associated preferentially with desmoid-type fibromatosis type and contributes to increased negative outcome in breast carcinoma (59).

Staining for CD10 can be scored as negative (0; no staining), weak (1; diffuse light staining or strong dark staining in less than 30% stromal cells) (**Figure 6**), strong (2; strong dark staining in more than 30% stromal cells) (**Figure 7**) (58)

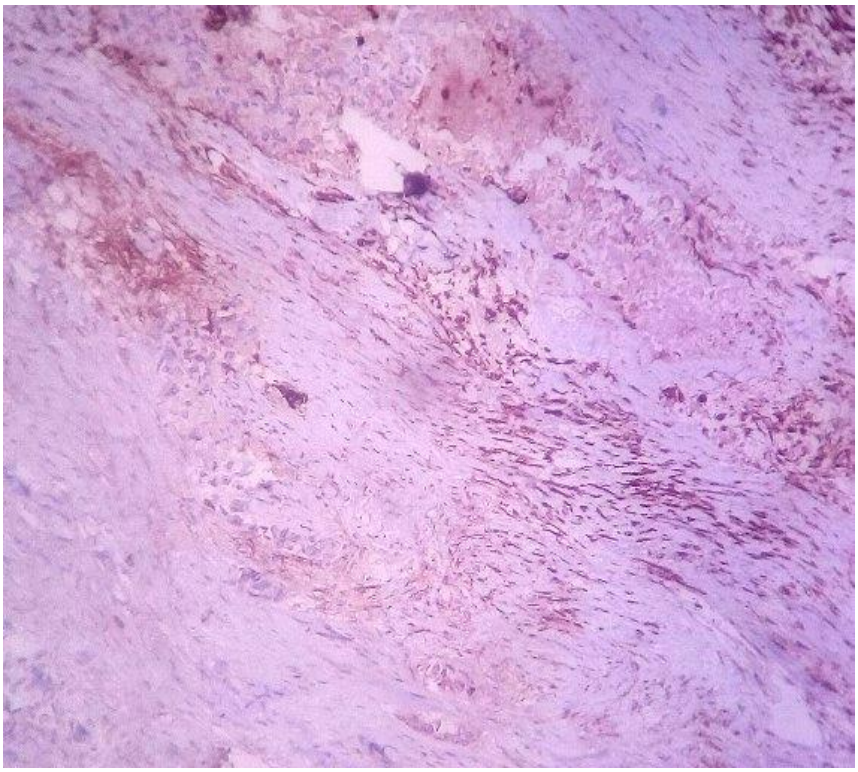


Figure 6 :CD10 weak positivity

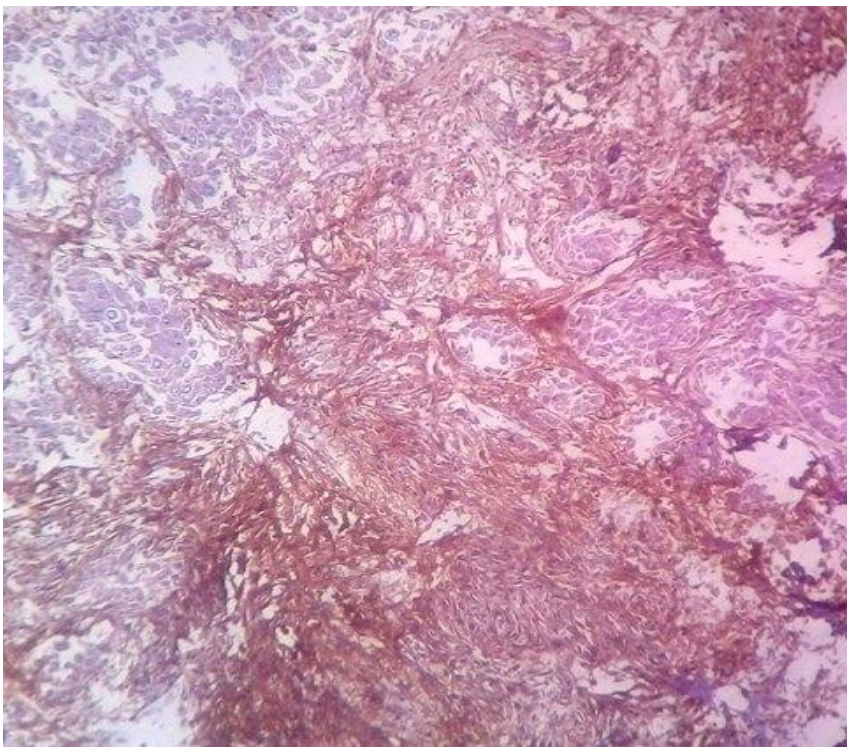


Figure 7 : CD10 strong positivity

SCORE	CD10 STAINING
Negative	<10% stromal positive cells/core
Weak	10-30% stromal positive cells/core
Strong	>30% stromal positive cells/core

Table 6 : CD10 intensity

Although CD10 has been very useful in classifying acute leukemias and subclassifying malignant lymphomas, it is also frequently expressed in renal cell carcinoma, hepatocellular carcinoma, carcinoma of urinary bladder and prostate (62). Renal cell carcinoma is selected as a control for this study (**Figure 8**).

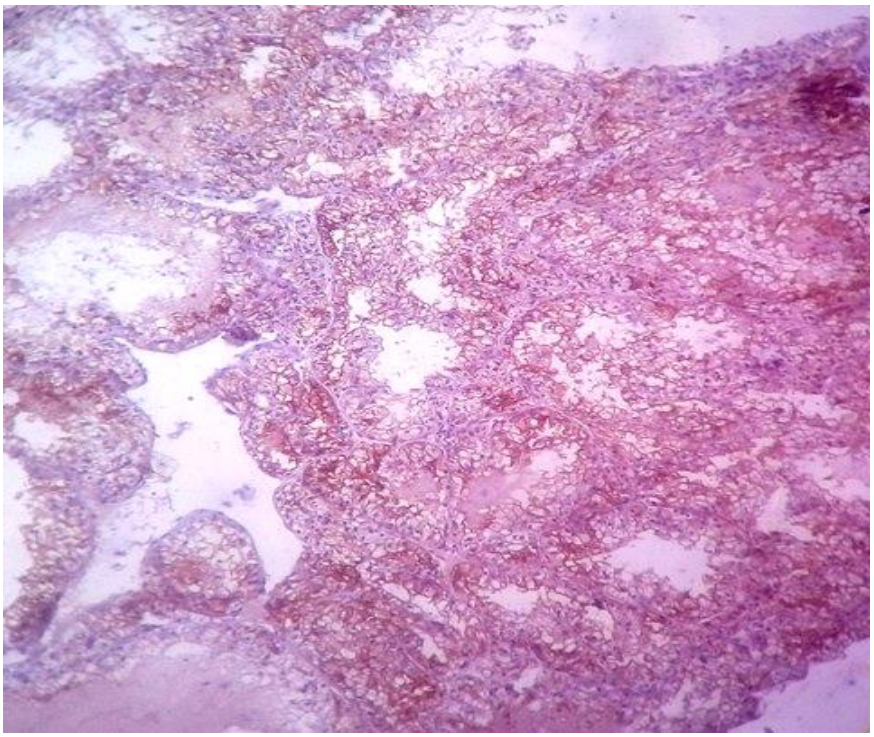


Figure 8 : CD10 control (renal cell carcinoma)

Molecular pathways:

FIVE MAJOR PATTERNS OF GENE EXPRESSION IN NST GROUP:

1. Luminal A (40% to 55% of NST cancers):
    - ER positive, HER2 neu negative
    - Majority are well or moderately differentiated, occur in postmenopausal women
    - Slow growing, respond well to hormonal treatment
    - But only a small number will respond to standard chemotherapy
2. Luminal B (15% to 20% of NST cancers):
    - ER positive, also often HER2 neu positive
    - Usually high grade
    - Responds less to hormonal therapy, but better to chemotherapy
3. Normal breast–like (6% to 10% of NST cancers):
    - ER positive, HER2 neu negative
    - Usually well differentiated
4. Basal-like (13% to 25% of NST cancers):
    - Triple negative
    - Express basal markers(basal keratins, p63, P-cadherin, laminin)

- Includes medullary carcinoma, metaplastic carcinoma, carcinomas with central fibrotic focus
- Aggressive with poor prognosis
- 15-20% respond to chemotherapy

5. HER2 positive (7% to 12% of NST cancers):
    - ER negative but HER2 neu overexpressed
    - overexpression is due to amplification of the segment of DNA on 17q21
    - Poorly differentiated, associated with high degree of brain metastasis

MOLECULAR CLASSIFICATION OF TRIPLE NEGATIVE BREAST CANCER:

- Basal like 1
- Basal like 2
- Immunomodulatory
- Mesenchymal
- Mesenchymal stem-like and
- Luminal androgen receptor types

	Key features	**Aberrant pathways**	**Potential targeted therapy**
Basal like 1	High Ki67 (average 70%) High PCR rate	Cell cycle DNA replication	Antimitotic agent-taxanes DNA-PK inhibitors TORC inhibitors
Basal like 2	High Ki67 (average 70%) High PCR rate	EGF/NGF/MET/ VVNT/IGF1:R	Antimitotic agent-taxanes Growth factor receptor inhibitors
Mesenchymal/ mesenchymal stem-like	Metaplastic carcinoma MSL, low level of	ECM/EMT Pathway involved in cell motility	P13K/mTOR inhibitors

	proliferation,low expression of claudins, and enrichment of stem cells		
Immuno modulatory	Medullary carcinoma	Immune signaling	PAPP inhibitors
Luminal androgen receptor	High AR and luminal cytokeratin(CK18) expression	AR pathway	Androgen antagonists

Table 7 : Molecular classification of triple negative breast cancer

Sayantan H.Jana et al. studied the expression of CD10 in 70 cases of breast cancer including 69 cases of radical mastectomy and 1 case of trucut biopsy and concluded that **CD10 stromal expression was significantly associated with increasing mitotic rate and tumor grade, worsening prognosis, ER negativity, HER2neu positivity** and with molecular subtypes (CD10 positivity with HER2 type and CD10 negativity with luminal type) (56)

Ali Taghizadeh-Kermani et al studied the stromal expression of CD10 in 50 patients of fibroadenoma and 100 patients of breast cancer out of which 28 percent were positive and concluded that **CD10 stromal expression significantly correlated with increasing tumor size and histological grade, presence of nodal metastases and ER negativity** (57)

Vandana Puri et al studied the expression of CD10 in 50 patients with breast carcinoma out of which 40 were positive and found that it correlated strongly with HER2neu and Ki67 positivity, ER/PR negativity and higher tumor grade (58)

Nikita A Makretsov et al studied the stromal CD10 expression using **tissue microarray method** in invasive breast carcinoma including 438 invasive carcinoma cases and 15 cases of DCIS and correlated that its expression is **associated with ER negativity, high tumor grade and decreased survival** (59)

Thomas S. Babu RJ et al studied the **effect of neo-adjuvant anthracycline based chemotherapy on stromal CD10 expression** in samples of breast cancer. Patients with invasive breast carcinoma scheduled for anthracycline based neoadjuvant chemotherapy were included. Out of 29 patients studied, 16 had strong CD10 expression. In these 16 patients, 14 were negative for hormonal receptors and 14 had HER2neu over expression. CD10 expression was studied post

chemotherapy and it remained the same in 13 cases, decreased in 13 cases and increased in 3 cases. Out of 13 cases where CD10 expression was decreased, 12 had clinical response. So it was concluded that **CD10 stromal expression correlated with hormone receptor negativity and HER2neu over expression** (60)

Maha E. Salama et al studied the expression of CD10 in desmoplastic stroma of breast carcinoma and neoplastic stroma of phyllodes tumor.70 cases were studied including 36 cases of invasive ductal carcinoma and 34 cases of phyllodes tumor. Positive expression was noted in 77.8% cases of invasive ductal carcinoma and 32.4% cases of phyllodes tumor. It was concluded that high level of **CD10 expression was correlated with malignant phyllodes and grade III breast cancer** (61)

Keiichi Iwaya et al studied the stromal expression of CD10 in 123 cases of carcinoma breast including 13 non invasive and 110 invasive breast carcinoma cases. CD10 expression was noted in 20 out of 110 invasive breast carcinoma cases and negative in all non invasive cases. Cases were followed up for a median period of 8 years and univariate and multivariate analysis were performed to evaluate prognostic significance of stromal CD10 expression. CD10 remained a significant predictor for time of recurrence in multivariate analysis (62)

IMMUNOHISTOCHEMISTRY

Immunohistochemistry involves two disciplines, immunology and histology. Immunohistochemistry is used to determine expression of particular antigen and its microanatomic location in the tissue.

Immunohistochemistry was started in 1940 when Coons developed an immunofluorescence technique to detect corresponding antigen in frozen sections.

Taylor and colleagues in 1974 showed it was possible to demonstrate antigens in routinely processed tissue. Antigen retrieval technique was introduced by Shi and associates in 1991. Antigen retrieval technique is a simple method that involves paraffin processed sections at high temperature before IHC staining.

The use of antibody in IHC depends on the sensitivity and specificity of the antigen –antibody reaction.

Blocking non-specific background staining

Background staining is due to either non specific binding or presence of endogenous enzymes. Non specific binding with polyclonal primary antibody is minimized by pre incubating sections with serum from same species on optimal working dilution.

Endogenous enzymes such as peroxidase seen in normal and neoplastic tissues is abolished by peroxidase blocking or by using alternate systems such as immunogold technique.

Methods suggested to overcome endogenous activity include incubation in methanol containing 0.5% hydrogen peroxide for 10 minutes at room temperature (almost complete abolition of endogenous peroxidase activity). Endogenous alkaline phosphatase is blocked by addition of 0.1M concentration of levamisole to the enzyme substrate solution.

Detection systems:

Antibodies are labeled or flagged by some method to permit visualization. These includes fluorescent substances, enzymes forming colored reaction with suitable substrate (light microscopy) or heavy metals(electron microscopy)

Methods of IHC:

Direct labeling method:

Antibody is attached with a label by chemical means and directly applied to tissue sections. It is a rapid and easy procedure and carries the disadvantage of using multiple antigens which require seperate incubation with respective antibodies.

Indirect labeling method:

Enzymes are labeled with secondary antibody, which is produced against primary antibody. This method is more sensitive and easy to handle. The advantages also include increased versatility, higher working dilution of primary antibody, secondary antibodies against primary antibodies of different species and easy to prepare.

Avidin biotin techniques:

High affinity binding between biotin and avidin is used in this procedure. Biotin is chemically linked to primary antibody and avidin is conjugated chemically to enzyme. The avidin binds to biotinylated antibody thus localizing the peroxidase moiety at the site of antigen.

Disadvantage of this technique is that, the endogenous biotin produces non specific background staining.

Avidin biotin conjugate procedure:

In this technique primary antibody is added followed by biotinylated secondary antibody and next by preformed complexes of avidin and biotin horse radish peroxidase conjugate. This is a more sensitive method.

Biotin streptavidin system:

Streptavidin is used in place of avidin. Streptavidin complexes are more stable.

Immunogold silver stain technique:

This is used in ultrastructural immunolocalisation. Gold particles are enhanced by the addition of several layers of metallic silver. The fine silver deposits in the background & creates confusion when small amounts of antigen are identified.

Polymeric method:

This technique permits binding of large number of enzyme molecules to a secondary antibody via the dextran backbone. Advantages of this technique are increased sensitivity, minimized non specific background staining and a reduction of total number of assay steps.

Tissue fixation, processing and antigen retrieval techniques:

Tissues for IHC undergo fixation, dehydration and paraffin embedding.

Fixation

This is a critical step, as the preservation of morphology is essential for interpretation of IHC.10% buffered formalin is commonly used because of the following advantages.

1. Good morphological preservation
2. Cheap
3. Prevents putrefactive changes in tissues
4. Carbohydrate antigens are better preserved.

The disadvantage of masking of antigens during fixation can be overcome by antigen retrieval techniques.

Antigen Retrieval:

This procedure involves unmasking of the antigens. Techniques are,

1. Proteolytic enzyme digestion
2. Microwave antigen retrieval
3. Microwave and trypsin and antigen retrieval technique.
4. Pressure cooker antigen retrieval.

MATERIALS AND METHODS

The study was done during the period January 2014-July 2015. It was carried in patients with confirmed diagnosis of carcinoma breast. The study was approved by ethical committee of Stanley Medical College.

STUDY POPULATION

CASES

The study sample comprised of 75 breast cancer patients. Cases were chosen from Department of General surgery, Stanley Medical College and Hospital. Age of the patient and histological grading was obtained for all cases. 75 patients were screened for receptor status of estrogen and progesterone as well as HER2neu and CD10 through immunohistochemical assay.

INCLUSION CRITERIA:

Patients with Infiltrating ductal carcinoma, NOS and its variants

EXCLUSION CRITERIA:

Patients with fibroadenoma, phyllodes tumor and other benign neoplasms.

METHOD OF DATA COLLECTION

75 cases were studied at random and selected for doing ER, PR, HER2neu and CD10 immunohistochemistry .The tissues so obtained were processed and sections were cut at 5 microns. Hematoxylin and eosin staining of the sections were done and studied.

METHOD OF TISSUE PREPARATION OF IHC

10% buffered formalin was used for fixing the specimens .The tissues were processed in various grades of alcohol and xylol using automated histokinette. Paraffin blocks were prepared and section of 5 micron thickness were cut in semiautomatic microtome using disposable blades and stained with hematoxylin and eosin. Suitable blocks were chosen for IHC.

Sections for immunohistochemistry were also cut in semiautomatic microtome using disposable blades. Slides coated with chrome alum were used. Sections were subjected to antigen retrieval using pressure cooker technique using TRIS EDTA (pH 9.2) buffer solution and then treated by HRP (horse radish peroxidase) polymer technique.

HRP POLYMER TECHNIQUE

The coated slides were taken through the following stages

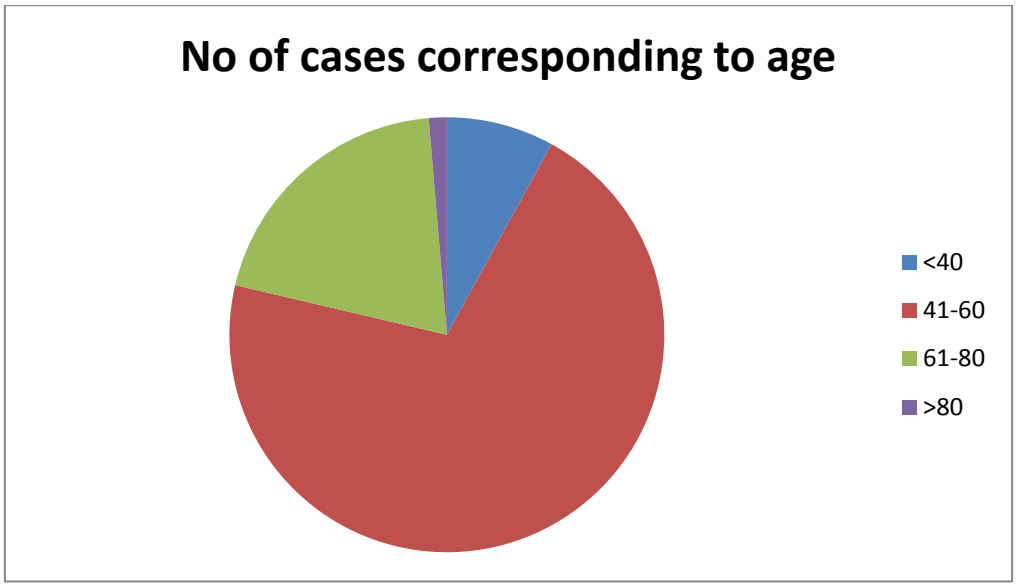
1. Overnight incubation (first at 70 degree Celsius for one hour, then at 40-45 degree Celsius for overnight)
2. Xylene- 2 changes, 15 minutes each
3. Graded alcohol- first with absolute alcohol- 2 wash, 5 minutes each. Then for 3 minutes with 90% alcohol and finally for 3 minutes with 70% alcohol
4. Distilled water rinse for 2-5 minutes
5. Antigen retrieval with pressure cooker
6. Wash with tap water gradually
7. TRIS buffer wash for 5 minutes, 2 changes
8. Treatment with peroxidase block –for inhibiting endogenous peroxidases in the tissue for 10 minutes
9. TRIS buffer wash for 5 minutes, 2 changes
10. Application of primary antibody for 45 minutes
11. TRIS buffer wash for 5 minutes, 2 changes
12. Application of superenhancer for 15 minutes which enhances the final reaction product by increasing the sensitivity of antigen antibody reaction.
13. TRIS buffer wash for 5 minutes, 2 changes

14. Application of SS label –secondary antibody from the goat with the tagged horse radish peroxidase enzyme for 15 minutes
15. TRIS buffer wash for 5 minutes, 2 changes
16. Application of DAB (Diaminobenzidine) chromogen for 5 minutes –this is cleaved by the enzyme to give the colored product at the antigen sites
17. Wash in distilled water for 5 minutes
18. The slides were counterstained with hematoxylin
19. Air dried and mounted with DPX (Distyrene dibutyl pthalide in Xylol)

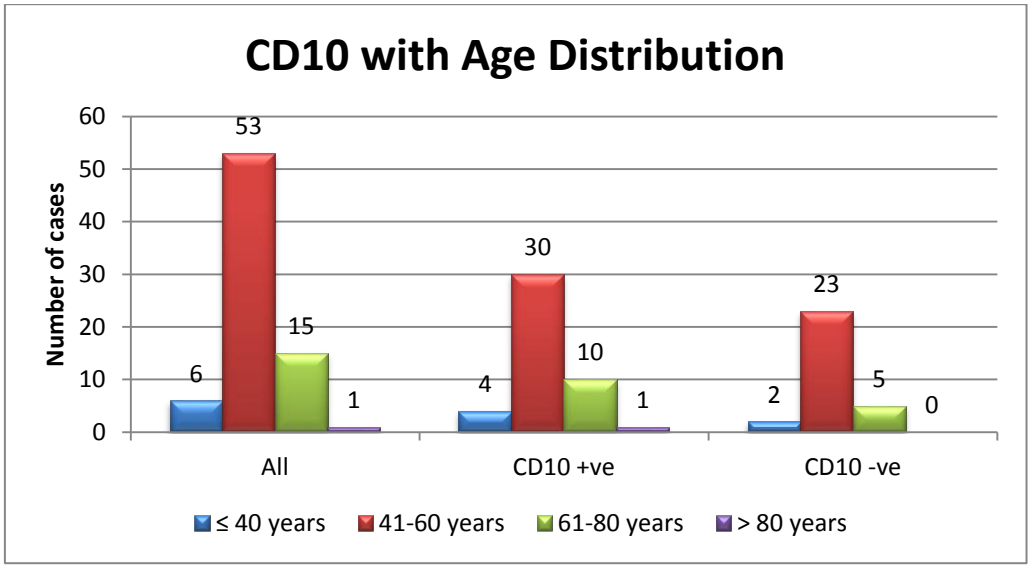
OBSERVATION AND RESULTS

In this study, we included 75 patients diagnosed with infiltrating ductal carcinoma fulfilling the inclusion and exclusion criteria.

1.Age



Graph 1 : Age wise distribution of number of cases



Graph 2 : Comparison of CD10 with age (All- No of cases)

Age Distribution	No. of cases	%	CD10 +ve	%	CD10 - ve	%
≤ 40 years	6	8.00	4	8.89	2	6.67
41-60 years	53	70.67	30	66.67	23	76.67
61-80 years	15	20.00	10	22.22	5	16.67
> 80 years	1	1.33	1	2.22	0	0.00
Total	75	100	45	100	30	100

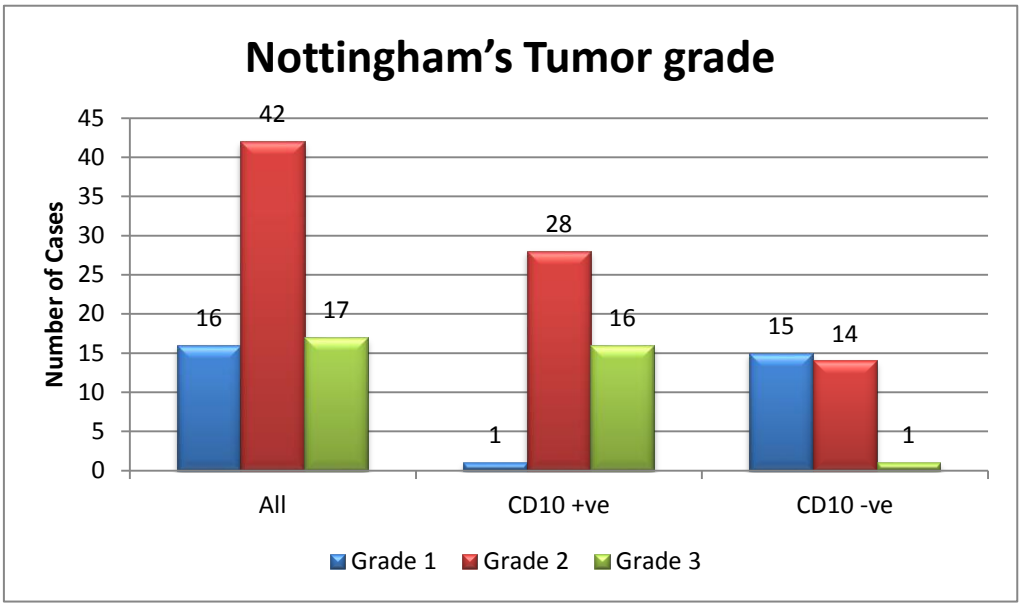
Table 8 : Age wise distribution of cases compared with CD10

Age Distribution	Number of cases	CD10 +ve	CD10 –ve
N	75	45	30
Mean	53.79	55.22	51.63
SD	10.64	11.47	9.00
P value Unpaired t Test			0.134671

Table 9 : P value for CD10 comparison with age

Out of 6 patients among those aged less than 40 years, 4 were CD10 positive and 2 were CD10 negative. In the age group between 40-60 years, out of 53 patients, 30 were CD10 positive and 23 were CD10 negative. In the age group of 61-80 years, out of 15 patients, 10 were CD10 positive and 5 were CD10 negative. In the age group of more than 80 years, 1 patient was included in the study and was CD10 positive.

2.Tumor grade



Graph 3 : Comparison of CD10 with histological grading (All- No of cases)

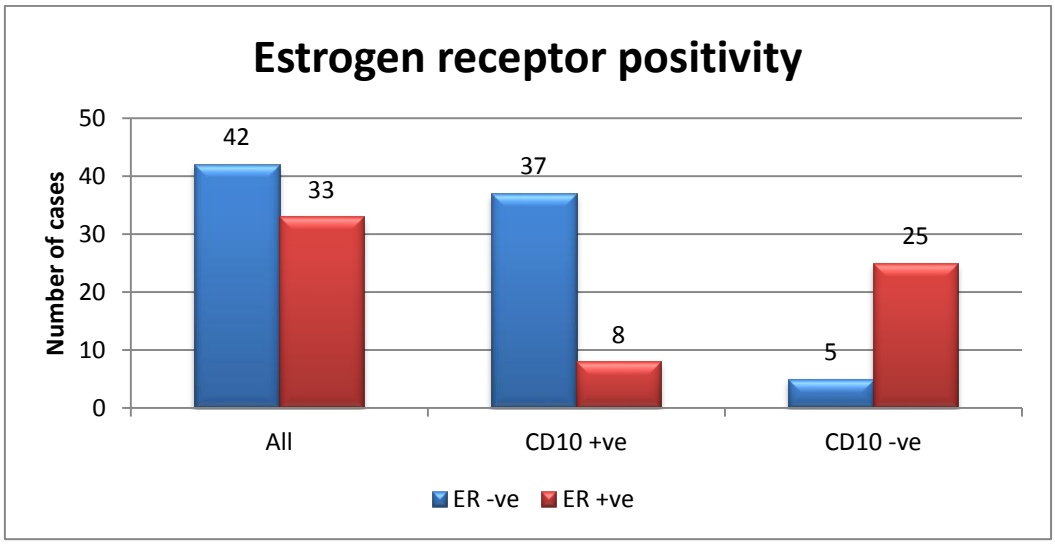
Nottingham's Tumor Grade	Number of cases	%	CD10 +ve	%	CD10 - ve	%
Grade 1	16	21.33	1	2.22	15	50.00
Grade 2	42	56.00	28	62.22	14	46.67
Grade 3	17	22.67	16	35.56	1	3.33
Total	75	100	45	100	30	100
P value Fishers Exact Test					< 0.0001	

Table 10 : P value for CD10 comparison with tumor grading

Out of 16 patients with histological grade, 1 was CD10 positive, 15 were CD10 negative. Out of 42 patients with histological grade 2, 28 were CD10 positive and 14 were CD10 negative. Out of 17 patients

with grade 3, 16 were CD10 positive and 1 was CD negative. The correlation was statistically significant (p value <0.0001)

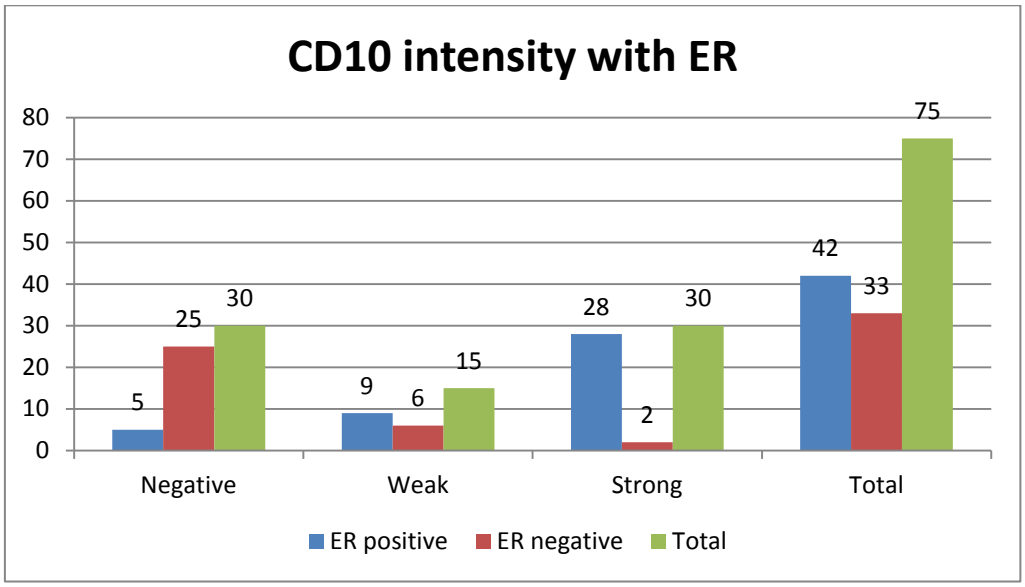
2. Estrogen receptor positivity



Graph 4 : Comparison of CD10 with ER status

Estrogen Receptor Positivity	Number of cases	%	CD10 +ve	%	CD10 - ve	%
ER -ve	42	56.00	37	82.22	5	16.67
ER +ve	33	44.00	8	17.78	25	83.33
Total	75	100	45	100	30	100
P value Fishers Exact Test					< 0.0001	
Sensitivity					24.2	
Specificity					11.9	
PPV					17.8	
NPV					16.7	

Table 11 : P value for CD10 comparison with ER status



Graph 5 : Comparison of CD10 intensity with ER

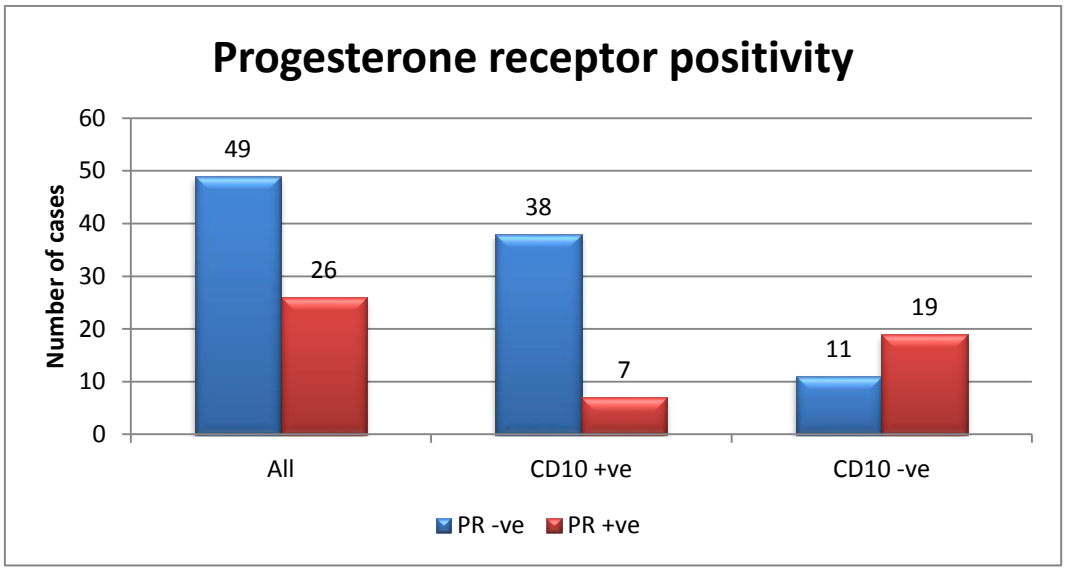
CD10	ER negative	ER positive	Total
Negative	5	25	30
Weak	9	6	15
Strong	28	2	30
Total	42	33	75

Table 12 : Comparison of CD10 intensity with ER

Out of 75 cases of breast carcinoma, 42 were ER negative and 33 were CD10 positive. Out of 42 ER negative cases, 37 were CD10 positive (9 were weakly stained and 33 had strong staining) and 5 were CD10 negative. Out of 33 ER positive cases, 8 were CD10 positive (6

were weakly stained and 2 had strong staining) and 25 were CD10 negative. The correlation was statistically significant (p value<0.0001)

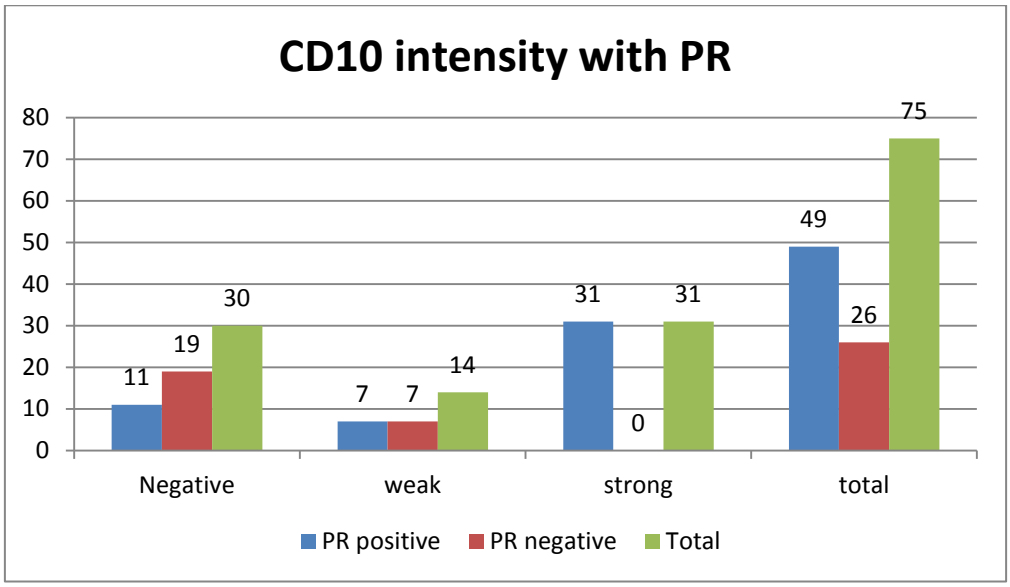
3. Progesterone receptor positivity



Graph 6 : Comparison of CD10 with PR status

Progesterone Receptor Positivity	Number of cases	%	CD10 +ve	%	CD10 - ve	%
PR -ve	49	65.33	38	84.44	11	36.67
PR +ve	26	34.67	7	15.56	19	63.33
Total	75	100	45	100	30	100
P value Fishers Exact Test					< 0.0001	
Sensitivity					26.9	
Specificity					22.4	
PPV					15.6	
NPV					36.7	

Table 13 : P value for Comparison of CD10 with PR status



Graph 7 : Comparison of CD10 intensity with PR

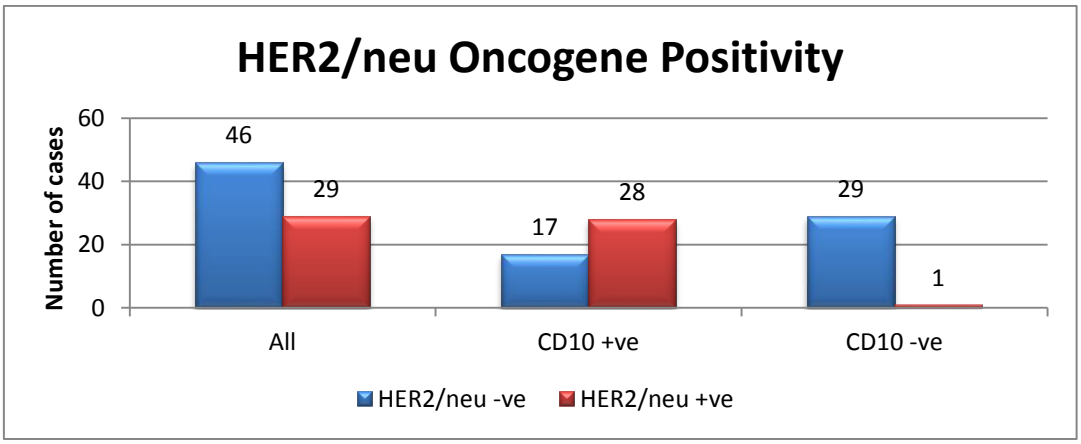
CD10	PR negative	PR positive	Total
Negative	11	19	30
Weak	7	7	14
Strong	31	0	31
Total	49	26	75

Table 14 : Comparison of CD10 intensity with PR

Out of 75 cases of carcinoma breast, 49 were PR negative and 26 were PR positive. Out of 49 PR negative cases, 38 were CD10 positive (7 were weakly stained and 31 had strong staining) and 11 were CD10 negative. Out of 26 PR positive cases, 7 were CD10 positive (all 7

cases weakly stained) and 19 were CD10 negative. The correlation was statistically significant (p value <0.0001)

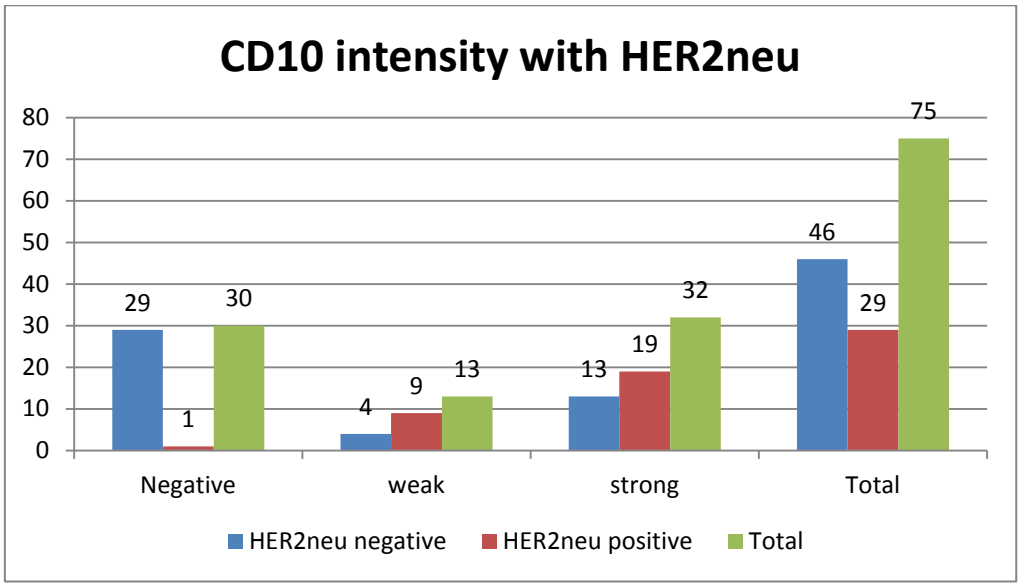
4. HER2neu oncogene positivity



Graph 8 : Comparison of CD10 with HER2neu status

HER2/neu Oncogene Positivity	Number of cases	%	CD10 +ve	%	CD10 – ve	%
HER2/neu - ve	46	61.33	17	37.78	29	96.67
HER2/neu +ve	29	38.67	28	62.22	1	3.33
Total	75	100	45	100	30	100
P value Fishers Exact Test					< 0.0001	
Sensitivity					96.6	
Specificity					63	
PPV					62.2	
NPV					96.7	

Table 15 : Comparison of CD10 with HER2neu status



Graph 9 : Comparison of CD10 intensity with HER2neu

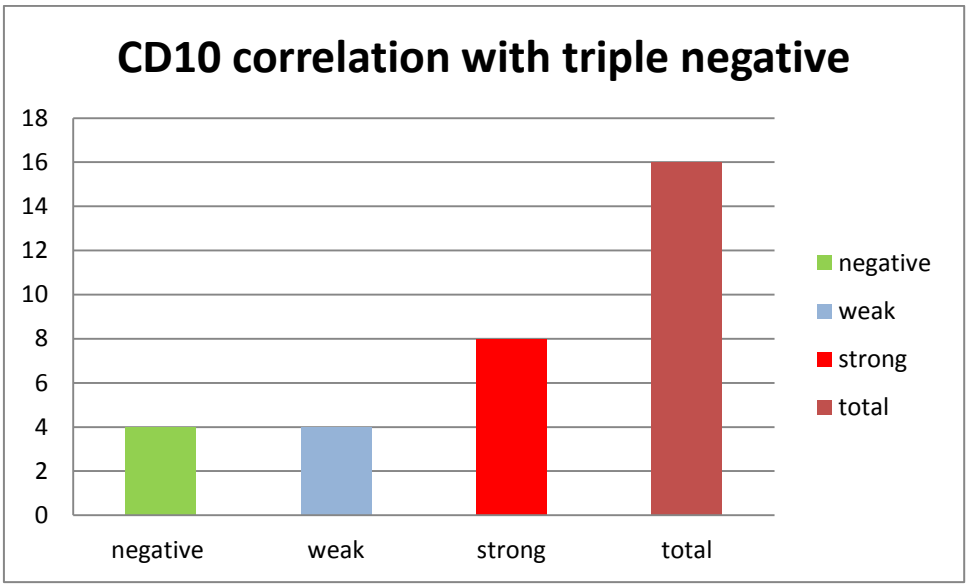
CD10	HER2neu negative	HER2neu positive	Total
Negative	29	1	30
Weak	4	9	13
Strong	13	19	32
Total	46	29	75

Table 16 : Comparison of CD10 intensity with HER2neu

Out of 75 cases of carcinoma breast, 46 were HER2neu negative and 29 were HER2neu positive. Out of 46 HER2neu negative cases, 17 were CD10 positive (4 were weakly stained and 13 had strong staining) and 29 were CD10 negative. Out of 29 HER2neu positive cases, 28 were CD10 positive (9 were weakly stained and 19 had strong staining)

and 1 was CD10 negative. The correlation was statistically significant (p value <0.0001)

5. Comparison of CD10 with triple negative status



Graph 10 : Comparison of CD10 with triple negative cases

Out of 16 triple negative (ER, PR and HER2neu negative) cases, 12 were CD10 positive and 4 were CD10 negative. Out of 12 CD10 positive cases, 8 were strongly stained and 4 had weak staining.

DISCUSSION

1.Age distribution

The age group of patients included in our study varied from less than 40 to more than 80 years with most of the patients belonging to 41-60 years (**Graph 1**). Mean age was 54 years (**Table 2**)

Sayantan et al in the year 2014 conducted a study which included patients with age less than 40 to more than 60 years (56)

A study conducted by Vandana puri et al in the year 2011 included patients from 30 to 80 years with a mean age of 48.5 years (58)

In the year 2013, a study conducted Thomas S Babu RJ et al included patients from 34 to 55 years with a mean age of 45 years (60)

2.Comparison of CD10 with age

In this present study, 53 out of 75 patients (70.67%) belonged to age group 41-60 years (**Table 8**). Out of 53 patients, 30 were CD10 positive and 23 were CD10 negative. Number of positive cases increased as the age advances but as overall when comparing patients of all age groups, comparison with CD10 positivity was not statistically significant (P value – 0.134671) (**Table 9**)

Sayantan H Jana et al in the year 2014 observed in his study that comparison of age with CD10 positivity was not statistically significant (P value- 0.3572) (56)

In the year 2014, a study done by Ali Taghizadeh-Kermani et al, 67 out of 100 cases of invasive carcinoma belonged to age group 41-60 years and comparison of age with CD10 positivity was not statistically significant (P value- 0.21) (57)

Name of study	**Age group**	**CD10 Positivity**	**P value**
Present study	41-60 years	30 out of 53	0.134671
Sayantan H Jana et al	40-60 years	19 out of 45	0.3572
Ali Taghizadeh-kermani et al	41-60 years	44 out of 67	0.21

3.Comparison of CD10 with histological grading

In the present study, out of 75 cases, 16 (21.33%) cases belonged to grade 1, 42 (56%) cases belonged to grade 2 and 17 (22.67%) cases belonged to grade 3 (**Graph 3**). Out of 17 grade 3 cases, 16 (35.56%)

were CD10 positive. The comparison between grade and CD10 was statistically significant (<0.0001) (**Table 10**)

In the year 2014, a study was conducted by Sayantan et al. It was observed that CD10 comparison was statistically significant (P value- 0.0413). Out of 22 cases which belonged to grade 3, 13 were CD10 positive (56)

Ali Teghizadeh-Kermani et al in the year 2014 observed in his study that comparison of CD10 positivity with higher histological grade (Grade 3) was statistically significant (P value<0.001). Out of 28 cases belonging to grade 3, 26 were CD10 positive out of which 15 were strongly stained (57)

A study conducted by Vandana Puri et al in the year 2011 in which most of the patients (26/49- 53.06%) belonged to grade 3 (58)

In the year 2007, a study was done by Nikita A Makretsov et al in which 68 patients belonged to grade 3 (26.4%) out of which 62 were CD10 positive and comparison with CD10 was statistically significant (P value- 0.02) (59)

In the year 2015 in a study conducted by Maha E. Salama et al, 7 cases were CD10 positive out of which 5 (45.5%) were strongly positive and 2 (18.2%) were weakly positive (61)

Keiichi Iwaya et al did a study in the year 2002 in which 22 patients belonged to grade 3 out of which only 3 cases were CD10 positive. Comparison with CD10 was not statistically significant (P value- 0.488) (62)

Name of study	**Grade 3 cases**	**CD10 positivity**	**P value**
Present study	17	3	<0.0001
Sayantan H Jana et al	22	13	0.0413
Ali Taghizadeh-Kermani et al	28	26	<0.001
Nikita A Makretsov	68	62	0.02
Keiichi Iwaya et al	22	3	0.488

4.Comparison of CD10 with ER status

In the present study, out of 75 cases, 42 cases were ER negative and 33 cases were ER positive (**Graph 4**). Out of 42 cases, 37

(82.22%) were CD10 positive and 5 (16.67%) cases were CD10 negative. Out of 33 ER positive cases, 8 (17.78%) were CD10 positive and 25 (83.33%) cases were CD10 negative. Comparison of CD10 positivity with ER negativity was statistically significant (P value <0.0001) with a sensitivity of 24.2% and specificity of 11.9% (**Table 11**)

Sayantan H Jana et al in the year 2014 did a study with 70 cases of breast carcinoma out of which 37 cases were ER negative and 33 cases were ER positive. Out of 37 ER negative cases, 26 (70%) were CD10 positive and 11 (30%) were CD10 negative. Out of 33 ER positive cases, 8 were CD10 (24%) positive and 25 (76%) were CD10 negative. Comparison of CD10 positivity with ER was statistically significant (P value- 0.0001). Sensitivity of the test was 77% and specificity was 70% (56)

In the year 2014, Ali Taghizadeh-Kermani observed in his study that out of 100 patients of invasive carcinoma of breast, 36 were ER negative and 64 were ER positive. Out of 36 ER negative cases, 6 were CD10 negative and 30 were CD10 positive. Out of 64 ER positive cases, 30 were CD10 negative and 34 were CD10 positive. Comparison of CD10 positivity with ER was statistically significant (P value- 0.003) (57)

Vandana Puri et al did a study in the year 2011 with 50 cases of carcinoma breast and observed that 35 cases were ER negative and 15 cases were ER positive. Out of 35 negative cases, 28 were CD10 positive but the comparison was not statistically significant (P value- 0.188). (58)

Nikita A Makretsov et al observed in the year 2007 in his study out of 438 cases, of invasive carcinoma, comparison of CD10 positivity with ER negativity was statistically significant (P value- 0.002) (59)

In the year 2013, Thomas S, Babu RJ et al observed in his study that CD10 positivity correlated with hormone receptor negativity (60)

Name of study	**ER negative cases**	**CD10 positivity**	**P value**
Present study	42	37	<0.0001
Sayantan H Jana et al	37	26	0.0001
Ali Taghizadeh-Kermani et al	36	30	0.003
Vandana Puri et al	35	28	0.188

5.Comparison of CD10 intensity with ER

In this present study, out of 42 ER negative cases, CD10 was negative in 5 and positive in 37 cases (**Graph 5**) (9 were weakly stained and 28 were strongly stained) (**Table 12**). So, CD10 positivity and intensity correlated strongly with increasing ER negativity.

In the year 2014, Ali Teghizadeh-Kermani et al observed that the intensity of CD10 staining increased with negativity of ER and the intensity decreased with increasing positivity of ER. Out of 36 ER negative cases, 6 (16.7%) were CD10 negative, 14 (38.9%) had weak CD10 staining and 16 (44.4%) had strong staining for CD10 (57)

Vandana Puri et al observed in the year 2011 that out of 35 ER negative cases, 7 were CD10 negative and 28 were CD10 were CD10 positive. Out of 28 CD10 positive cases, 7 were weakly stained and 21 were strongly stained, so the intensity of CD10 staining increased with increasing ER negativity (58)

In the year 2013, Thomas S, Babu RJ et al did a study on expression of CD10 and hormone receptors post anthracycline treated patients and compared it with pre-chemotherapy results. Before chemotherapy, CD10 positivity was observed in 24 cases and negative in 5 cases. Out of 24 positive cases, 16 were strongly stained and 8 were weakly stained. Out of 16 strongly positive CD10 cases, 14 were

negative for hormonal receptors. After chemotherapy, CD10 stromal expression remained same in 13 cases with change of expression in remaining 16 cases (CD10 expression decreased in 13 cases and increased in 3 cases). So CD10 expression and its intensity reduced post chemotherapy since 20 out of 24 positive (pre-chemotherapy) patients responded to therapy (60)

Maha E.Salama et al did a study in the year 2015 on 70 cases including 36 cases of invasive duct carcinoma and 34 cases of phyllodes tumor. CD10 expression was observed in 28 cases of invasive ductal carcinoma out of which 50% of grade 1 cases showing negative CD10 expression with 33.3% showing expression whereas CD10 was positive in most of grade 3 cases with most showing strong staining (61)

Name of study	**ER negative cases**	**CD10 positivity (weak)**	**CD10 positivity (strong)**	**P value**
Present study	42	9	28	<0.0001
Ali Taghizadeh-Kermani et al	36	14	16	0.003
Vandana Puri et al	35	7	21	0.188

6.Comparison of CD10 with PR status

In the present study, out of 75 cases, 49 cases were PR negative and 26 cases were PR positive (**Graph 6**). Out of 49 PR negative cases, 38 (84.44%) were CD10 positive and 11 (36.67%) cases were CD10 negative. Out of 26 PR positive cases, 7 (15.56%) were CD10 positive and 19 (63.33%) cases were CD10 negative. Comparison of CD10 positivity with PR negativity was statistically significant (P value <0.0001) with a sensitivity of 26.9% and specificity of 22.4% (**Table 13**)

Sayantan H Jana et al did a study in the year 2014 with 70 cases of breast carcinoma out of which 50 cases were PR negative and 20 cases were PR positive. Out of 50 PR negative cases, 27 (54%) were CD10 positive and 23 (46%) were CD10 negative. Out of 20 PR positive cases, 7 were CD10 (35%) positive and 13 (65%) were CD10 negative. Comparison of CD10 positivity with PR was not statistically significant (P value- 0.1902). Sensitivity of the test was 21% and specificity was 64% (56)

In the year 2014, Ali Taghizadeh-Kermani observed in his study that out of 100 patients of invasive carcinoma of breast, 46 were PR negative and 54 were PR positive. Out of 46 PR negative cases, 14 were CD10 negative and 32 were CD10 positive. Out of 54 PR positive

cases, 22 were CD10 negative and 32 were CD10 positive. Comparison of CD10 positivity with PR was not statistically significant (P value-0.07) (57)

Nikita A Makretsov et al observed in their study in the year 2007 that out of 438 cases, of invasive breast carcinoma, comparison of CD10 positivity with PR negativity was not statistically significant (P value->0.05) (41)

In the year 2013, Thomas S, Babu RJ et al observed in his study that CD10 positivity correlated with hormone receptor negativity (60)

Name of study	**PR negative cases**	**CD10 positivity**	**P value**
Present study	49	38	<0.0001
Sayantan H Jana et al	50	27	0.1902
Ali Taghizadeh-Kermani et al	46	32	0.07

7.Comparison of CD10 intensity with PR

In this present study, out of 49 PR negative cases, CD10 was negative in 11 and positive in 38 cases (**Graph 7**) of which 7 were weakly stained and 31 had strongly staining (**Table 14**). So CD10 positivity and intensity correlated strongly with increasing PR negativity.

In the year 2014, Ali Teghizadeh-Kermani et al observed that the intensity of CD10 staining increased with negativity of PR and the intensity decreased with increasing positivity of PR. Out of 46 PR negative cases, 14 (30.4%) were CD10 negative, 22 (47.8%) had weak CD10 staining and 10 (21.7%) had strong staining for CD10. (57)

Name of study	**PR negative cases**	**CD10 positivity (weak)**	**CD10 positivity (strong)**	**P value**
Present study	49	7	31	<0.0001
Ali Taghizadeh-Kermani et al	46	22	10	0.07

8.Comparison of CD10 with HER2neu status

In the present study, out of 75 cases, 46 cases were HER2neu negative and 29 cases were HER2neu positive (**Graph 8**). Out of 46 negative cases, 17 (37.78%) were CD10 positive and 29 (96.67%) cases were CD10 negative. Out of 29 HER2neu positive cases, 28 (62.22%) were CD10 positive and 1 (3.33%) case was CD10 negative. Comparison of CD10 positivity with HER2neu positivity was statistically significant (P value <0.0001) with a sensitivity of 96.6% and specificity of 63% (**Table 15**)

Sayantan H Jana et al in the year 2014 did a study with 70 breast carcinoma cases out of which 25 cases were HER2neu negative and 45 cases were HER2neu positive. Out of 25 HER2neu negative cases, 18 (72%) were CD10 positive and 7 (28%) were CD10 negative. Out of 45 HER2neu positive cases, 16 were CD10 (35%) positive and 29 (65%) were CD10 negative. Comparison of CD10 positivity with HER2neu was statistically significant (P value- 0.0057). Sensitivity of the test was 53% and specificity was 80% (56)

Vandana Puri et al observed in their study in the year 2011 that out of 20 HER2neu negative cases, 8 were CD10 negative and 12 were CD10 positive. Out of 30 HER2neu positive cases, 2 were CD10

negative and 28 were CD10 positive. There was positive statistical significance between HER2neu and CD10 positivity (P value- .000) (58)

In the year 2011, Vandana Puri et al did a study with 50 cases of carcinoma breast and observed that 10 cases were HER2neu negative and 40 cases were HER2neu positive and concluded that HER2neu positivity increased with increasing positivity of CD10 (58)

Nikita A Makretsov et al observed in the year 2007 in his study out of 438 cases, of invasive breast carcinoma, comparison of CD10 positivity with HER2neu was not statistically significant (59)

In the year 2013, Thomas S, Babu RJ et al observed in his study that out of 29 cases, 20 were hormone receptor negative, 23 had HER2neu overexpression and CD10 positivity was noted in 24 cases (16 cases were strongly stained and 8 cases had weak staining) before administration of chemotherapy. On correlating HER2neu with CD10 expression, out of 16 strongly positive CD10 cases, 14 had HER2neu overexpression (60)

Name of study	HER2neu negative cases	CD10 positivity	P value
Present study	46	17	<0.0001
Sayantan H Jana et al	25	18	0.0057
Vandana Puri et al	20	12	0.000

9.Comparison of CD10 intensity with Her2neu

In this present study, out of 46 HER2neu negative cases, CD10 was negative in 29 and positive in 17 cases (**Graph 9**) of which 4 were weakly stained and 13 were strongly stained (**Table 16**). Out of 29 HER2neu positive cases, 28 were CD10 positive. So CD10 positivity and intensity correlated strongly with increasing HER2neu positivity.

Vandana Puri et al observed in their study in the year 2011 that out of 20 HER2neu negative cases, 8 were CD10 negative and 12 were CD10 positive. Out of 12 CD10 positive cases, 9 were weakly stained and 3 were strongly stained. Out of 30 HER2neu positive cases, 2 were CD10 negative and 28 were CD10 positive. Out of 28 CD10

positive cases, 7 were weakly stained and 21 were strongly stained. So there was increasing intensity of CD10 with increasing positivity of HER2neu (58)

In the year 2013, Thomas S, Babu RJ et al did a study on expression of CD10 and hormone receptors on post anthracycline treated patients and compared it with pre-chemotherapy results. Before chemotherapy, CD10 positivity was observed in 24 cases and negative in 5 cases. Out of 24 positive cases, 16 were strongly stained and 8 were weakly stained. Out of 16 strongly positive CD10 cases, 14 had HER2neu overexpression. After chemotherapy, CD10 stromal expression remained same in 13 cases with change of expression in remaining 16 cases (CD10 expression decreased in 13 cases and increased in 3 cases). So CD10 expression and its intensity reduced post chemotherapy since 20 out of 24 positive (pre-chemotherapy) patients responded to therapy (60)

Name of study	**HER2neu negative cases**	**CD10 positivity (weak)**	**CD10 positivity (strong)**	**P value**
Present study	46	4	13	<0.0001
Vandana Puri	20	9	3	0.000

10.Comparison of CD10 with triple negative status

In the present study, out of 75 cases included in the study, 16 were triple negative. Out of 16 triple negative cases, 12 were CD10 positive and 4 were CD10 negative. Out of 12 CD10 positive cases, 4 were weakly stained and 8 had strong staining. So there was correlation between triple negative cases and CD10 positivity and its intensity (**Graph 10**).

Sayantan H Jana et al observed in his study in the year 2014 that out of 70 patients included in the study, 17 were negative for ER,PR and HER2neu. Out of 17 triple negative cases, 11 (65%) were CD10 positive and 6 (35%) were CD10 negative. The comparison was statistically significant (P value- 0.0004) (56)

Name of study	**Triple negative cases**	**CD10 positivity**
Present study	16	12
Sayantan H Jana et al	17	11

CONCLUSION

This study was carried out in the Department of Pathology, Govt. Stanley Medical College, in collaboration with the Department of General surgery, Govt. Stanley Hospital. Total of 75 modified radical mastectomy specimens were taken for this study

- The stromal expression of CD10 has significant correlation with **higher histological grade** (**Grade 3**), **ER negativity**, **PR negativity** and **HER2neu positivity** and **triple negativity**
- There is no correlation between CD10 expression and age of the patient
- The intensity of CD10 positivity also increased with increasing histological grade, hormonal receptor negativity, HER2neu positivity and triple negative cases
- There is wide expression of CD10 in desmoplastic stroma of breast carcinoma and negative immunoreactivity in stromal cells of normal breast
- This study highlights the role of stromal CD10 expression in predicting tumor response and prognosis and therefore CD10 could be included as a routine marker along with other markers for invasive carcinoma breast before giving chemotherapy

BIBILIOGRAPHY

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C and Parkin DM.

   GLOBOCAN 2008 v2.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10 [Internet]. Lyon, France: International Agency for Research on Cancer; 2010. Available from: http://globocan.iarc.fr.
2. Jemal A et al; Cancer statistics 2008;CA cancer J clin 58:71,2008
3. David J.winchester,David P.Vinchester,M.D. et al;Breast Cancer;2nd edition;elsevier publication;2006;pg 1
4. WHO(2008).Global burden of disease: 2004 update;Death and DALYs 2004 Annex tables pg.56
5. Da louw,Dm van ede,Ae louw et al Human development ,2nd edition Pearson South Africa,2007 pg.389'
6. Toniolo PG, Levitz M, Zeleniuch-Jacquotte A, et al. A prospective study of endogenous estrogens and breast cancer in postmenopausal women. *J Natl Cancer Inst* 1995;**87**:190–7.
7. Feogelson HS,Ross RK,YU MC,Coetzee GA,Reichardt JK & Henderson BE.Genetic susceptibility to cancer from exogenous and

endogenous exposures.Journal of Cell Biochemistry.Supplement 1996 25 15-22

8. Christof sohn et al,Breast ultrasound: A systemic approach to technique image interpretation ,illustrated,Thieme publisher pg:1

9. Boyd NF,Martin LJ,Bronskill MJ,Duric N,Minkin S. Breast tissue composition and Susceptibility to Breast cancer.J Natl Cancer Inst 2010;102:1-14

10. Roger thanner this et al,Physiology an illustrated review,Thieme publisher,2011 pg:301

11. Michael S.Sabel et al Surgical foundations :Essentials of Breast Surgery 1 st edition Pg:16

12. Charite etal ,Hormone replacement therapy,breast cancer and tumour pathology ,Maturitas,2009,65(3):183-189

13. Freddie bray ,Peter Mc Carron ,Maxwell parkin D et al,The changing global patterns of female breast cancer incidence and mortality ,Breast Cancer Res 2004,6:229-239

14. Christopher Kwesi O,Williams,Olufunmalayo L,Olopase,Carla l.Falkson et al,Breast cancer in Women of African descent,Springer publication ;2006;pg 82

15. Imran Ali,Waseem A.Wani and Kishwar Saleem et al,Cancer Scenario in India with Future perspectives ,Cancer Therapy;2011;vol:8:pg 56-70.

16. Jerzy E.Tyczynski,Freddie Bray,Maxwell Parkin D et al,ENCR Cancer Fact sheets:ENCR,IARC,Vol: 2:December 2002

17. Vinay Kumar;Abdul K.Abbas et al; Robbins and Cotran Pathologic Basis of disease;9th edition;Sauder Elesiver Publications,pg 1051-52.

18. Christopher l-Fu-Li et al ,Breast Cancer Epidemiology,illustrated,springer publication;2010:pg 389

19. Guido Majno,Isabella Joris et al ,Cells,Tissues and disease:Principles of general pathology, second edition,Oxford university Press,2004:pg 951

20. Monica Morrow,MD,Virgil Craig Jordan et al ,Managing Breast cancer Risk,illustrated,PMP-USA;2003:pg 47

21. Edward C.Halperin,Carlos.A.Perez,Luther.W.Brady et al,Perez and Brady's Principles and Practice of Radiation Oncolgy;fifth edition;Lippincott Williams and Wilkins,2008;pg 1177

22. J.C.E.Underwood, S.S.Cross eta al;General and Systemic Pathology;5th edition;Churchill Livigstone Elesiver Publications,2002,pg-996-999

23. Cho E,Holmes M,Hankinson SE,Willett WC,Nutrients involved in one carbon metabolism and risk of breast cancer among premenopausal women.Cancer Epidemiol Biomarkers Prev.2007z;16:2787-2790

24. Shin MH,Holmes MD,Hankinson SE et al,Intake of dairy products,calcium,vitamin D and risk of breast cancer.J Natl Cancer Inst.2002;94:1301-1311

25. Fortunati.N,Catalano.M.G,Boccuzzi.G,Friaria.R et al Sex hormone binding globulin,estradio and breast cancer,Moecular and Celllar Endocrinology ;2010;vol 316:pg 86-92

26. David J.winchester,David P.Vinchester,M.D. et al;Breast Cancer;2nd edition;elsevier publication;2006;pg 55

27. The endogenous hormones and breast cancer collaborative group,Endogenous Sex hormones and Breast cancer in Post menopausal women:Reanalysis of nine prospective studies,J Natl Cancer Inst 2002:94(8):606-616

28. Daniel B.Kopans et al ,Breast imaging,Third edition,Lippincott Williams and Willkins ,2007,pg 89-90

29. Edward R.Sauter,Mary.B.Daly et al ,Breast cancer risk reduction and Early detection,Springer Publication ,2010: pg 6

30. Edward R.Sauter,Mary.B.Daly et al ,Breast cancer risk reduction and Early detection,Springer Publication ,2010: pg 9-10.

31. Norman.F.Boyd,Gina A.lockwood,Lisa J Martin,Julia A.Knight,Roberta A.Jong,Eve Fishell,Jeff W.Byng,Martin J.Yaffe and David L.Tritchler et al,Mammographic densities and Risk of Breast cancer among Subjects with a family history of this disease,J Natl Cancer Inst 1991;vol 16:pg 1404-1408

32. Charles Bruenicardi et al,Schwartz principles of surgery,9th edition;McGraw Hill Publications 2010:pg445-448

33. Victor G.Vogel,M.D,Therese Bevers et al ,Breast cancer Risk Assessment Evidence based Guidelines for evaluation ,Prevention ,Counselling,Treatement,illustratrated,Jones and Barlett learning,2003:pg

34. The International agency for research on cancer,World health Organization:Tumours of the Breast and Female genital organs,2002:pg 10

35. Josef.E.Fischer,K.I.Bland,Mark.P.Callery et al,Master of surgery ,5th edition, Lippincott williams and wilkins,2007;Vol 1:pg 493

36. Yi Huang,Nancy E.Davidson et al ,Principles of molecular medicine, second edition,2006,pg:728-735

37. Karin Dahlman-Wright et al,International union of pharmacology, LXIV. EstrogenReceptors, Pharmacological reviews, December 006,vol.58,pgno.4,773-781

38. Vollenweider-Zerargui L, Barrelet L, Wong Y, Lemarchand-Béraud T, Gómez F. The predictive value of estrogen and progesterone receptors' concentrations on the clinical behaviour of breast cancer in women. Clinical correlation on 547 patients. *Cancer*. 1986;57(6):1171–80.

39. Cui X, Zhang P, Deng W, et al. Insulin-like growth factor-I inhibits progesterone receptor expression in breast cancer cells via the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin pathway: progesterone receptor as a potential indicator

of growth factor activity in breast cancer. *Mol Endocrinol*. 2003;17(4):575–88.

40. Cui X, Schiff R, Arpino G, Osborne CK, Lee AV. Biology of progesterone receptor loss in breast cancer and its implications for endocrine therapy. *J Clin Oncol*. 2005;23(30):7721–35.

41. Jacquemier JD, Hassoun J, Torrente M, et al: Distribution of estrogen and progesterone receptors in healthy tissue adjacent to breast lesions at various stages–immunohistochemical study of 107 cases. *Breast Cancer Res Treat.* 15:109–117, 1990.

42. Coussens L, Yang-Feng TL, Liao YC, et al: Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene. *Science*. 230:1132–1139, 1985.

43. Semba K, Kamata N, Toyoshima K, et al: A v-erbB-related protooncogene, c-erbB-2, is distinct from the c-erbB-1/epidermal growth factor-receptor gene and is amplified in a human salivary gland adenocarcinoma. *Proc Natl Acad Sci U S A.* 82:6497–6501, 1985.

44. Di Fiore PP, Pierce JH, Kraus MH, et al: erbB-2 is a potent oncogene when overexpressed in NIH/3T3 cells. *Science*. 237:178–182, 1987.

45. Akiyama T, Sudo C, Ogawara H, et al: The product of the human c-erbB-2 gene: a 185-kilodalton glycoprotein with tyrosine kinase activity. *Science*. 232:1644–1646, 1986.
46. Slamon DJ, Clark GM, Wong SG, et al: Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science*. 235:177–182, 1987.
47. Owens MA, Horten BC, Da Silva MM: HER2 amplification ratios by fluorescence in situ hybridization and correlation with immunohistochemistry in a cohort of 6556 breast cancer tissues. *Clin Breast Cancer*. 5:63–69, 2004.
48. Yaziji H, Goldstein LC, Barry TS, et al: HER-2 testing in breast cancer using parallel tissue-based methods. *JAMA*. 291:1972– 1977, 2004.
49. Hayes DF, Thor AD, Dressler LG, et al: HER2 and response to paclitaxel in node-positive breast cancer. *N Engl J Med*. 357:1496–1506, 2007.
50. Konecny GE, Thomssen C, Luck HJ, et al: Her-2/neu gene amplification and response to paclitaxel in patients with metastatic breast cancer. *J Natl Cancer Inst*. 96:1141–1151, 2004.
51. Menard S, Valagussa P, Pilotti S, et al: Response to cyclophosphamide, methotrexate, and fluorouracil in lymph node-positive breast cancer according to HER2 overexpression and other tumor biologic variables. *J Clin Oncol*. 19:329–335, 2001.

52. Pritchard KI, Shepherd LE, O'Malley FP, et al: HER2 and responsiveness of breast cancer to adjuvant chemotherapy. *N Engl J Med*. 354:2103–2111, 2006.
53. Thor AD, Berry DA, Budman DR, et al: erbB-2, p53, and efficacy of adjuvant therapy in lymph node-positive breast cancer. *J Natl Cancer Inst*. 90:1346–1360, 1998.
54. Ellis MJ, Coop A, Singh B, et al: Letrozole is more effective neoadjuvant endocrine therapy than tamoxifen for ErbB-1- and/ or ErbB-2-positive, estrogen receptor-positive primary breast cancer: evidence from a phase III randomized trial. *J Clin Oncol*. 19:3808–3816, 2001.
55. Konecny G, Pauletti G, Pegram M, et al: Quantitative association between HER-2/neu and steroid hormone receptors in hormone receptor-positive primary breast cancer. *J Natl Cancer Inst*. 95:142–153, 2003.
56. Sayantan H. Jana, Bharti M. Jha, Chandni Patel, Dipan Jana, Anshul Agarwal. CD10- A new prognostic stromal marker in breast carcinoma, its utility, limitations and role in breast cancer pathogenesis. Indian Journal of Pathology and Microbiology – 57(4), October-December 2014, DOI: 10.4103/0377-4929.142639

57. Ali Taghizadeh-Kermani et al, The stromal overexpression of CD10 in Invasive Breast Cancer and its Association with Clinicopathologic factors. Iran J Cancer Prev. 2014; 1:17-21

58. Vandana Puri, Manjula Jain and Shaji Thomas. Stromal Expression of CD10 in Invasive Breast Carcinoma and Its Correlation with ER, PR, HER2neu and Ki67. International Journal of Breast Cancer. Volume 2011, Article ID 437957, 4 pages, doi:10.4061/2011/437957

59. Nikita A Makretsov et al, Stromal CD10 expression in invasive breast carcinoma correlates with poor prognosis, estrogen receptor negativity, and high grade. Modern Pathology (2007) 20, 84–89

60. Thomas S, Babu RJ, Agarwal K, Puri V, Jain M, Andley M, *et al*. Effect of neoadjuvant chemotherapy on stromal CD10 antigens in breast cancer - A preliminary study. Indian J Cancer 2013;50:46-51

61. Maha E. Salama, Wael S. Ibrahim and Dina A.Khairy. Comparison of CD10 expression in stroma of epithelial and mesenchymal tumors of the breast. Global Advanced Research Journal of Medicine and Medical Science (ISSN: 2315-5159) Vol. 4(1) pp. 051-056, January, 2015

62. Keiichi Iwaya et al, Stromal expression of CD10 in invasive breast carcinoma: a new predictor of clinical outcome. Virchows Arch (2002) 440:589–593. DOI 10.1007/s00428-002-0639-4

MASTER CHART

S.no	Age	Grading	ER	PR	HER2neu	CD10	Grading
1	80	2	neg	neg	pos	Neg	neg
2	44	3	neg	neg	neg	Pos	strong
3	60	2	pos	pos	neg	Neg	neg
4	45	3	neg	neg	neg	Pos	strong
5	58	2	neg	neg	pos	Pos	strong
6	48	1	pos	neg	neg	Neg	neg
7	30	3	neg	neg	pos	Pos	strong
8	55	2	neg	neg	pos	Pos	weak
9	48	2	neg	neg	neg	Pos	weak
10	60	1	pos	pos	neg	Neg	neg
11	55	2	pos	pos	pos	Pos	weak
12	50	3	neg	neg	neg	Neg	neg
13	62	2	neg	neg	neg	Pos	strong
14	59	3	neg	pos	pos	Pos	weak
15	51	2	pos	neg	neg	Neg	strong
16	49	3	pos	pos	pos	Pos	weak
17	50	2	neg	neg	neg	Neg	neg
18	55	3	neg	neg	pos	Pos	strong
19	43	2	neg	neg	neg	Neg	neg
20	60	2	neg	neg	pos	Pos	strong
21	65	2	neg	neg	pos	Pos	strong
22	50	2	neg	neg	neg	Pos	weak
23	44	2	pos	pos	neg	Neg	neg
24	50	2	neg	neg	neg	Pos	strong
25	70	2	pos	neg	neg	Neg	neg
26	68	2	pos	pos	pos	Pos	weak
27	45	3	neg	neg	neg	Pos	strong
28	40	2	pos	pos	pos	Pos	weak
29	53	2	neg	neg	pos	Pos	strong
30	51	2	neg	neg	pos	Pos	strong
31	62	2	pos	pos	neg	Neg	neg
32	55	1	pos	pos	neg	Neg	neg
33	60	2	pos	pos	neg	Pos	weak
34	95	2	pos	neg	neg	Pos	strong
35	46	2	neg	neg	neg	Pos	strong
36	69	3	neg	neg	pos	Pos	strong
37	48	1	pos	pos	neg	Neg	neg
38	30	1	pos	pos	neg	Neg	neg
39	65	1	pos	pos	neg	Neg	neg
40	47	2	pos	pos	neg	Neg	neg
41	60	1	pos	pos	neg	Neg	neg
42	56	2	neg	neg	pos	Pos	strong

43	48	2	pos	pos	neg	Pos	weak
44	42	1	pos	pos	neg	Neg	neg
45	34	3	neg	neg	neg	Pos	strong
46	46	1	pos	pos	neg	Neg	neg
47	45	2	neg	pos	pos	Neg	neg
48	60	3	neg	neg	pos	Pos	strong
49	52	1	pos	pos	neg	Neg	neg
50	60	2	neg	neg	neg	Pos	weak
51	54	1	pos	pos	neg	Neg	neg
52	47	2	pos	neg	neg	Neg	neg
53	63	2	neg	neg	pos	Pos	strong
54	57	2	neg	neg	pos	Pos	strong
55	56	3	neg	neg	neg	Pos	strong
56	49	1	neg	neg	neg	Neg	neg
57	40	3	neg	neg	neg	Pos	strong
58	40	2	pos	pos	neg	Neg	neg
59	64	2	neg	neg	pos	Pos	strong
60	45	2	pos	neg	pos	Pos	strong
61	57	2	neg	neg	neg	Pos	weak
62	62	2	pos	pos	neg	Neg	neg
63	43	1	neg	neg	pos	Pos	strong
64	60	3	neg	neg	pos	Pos	strong
65	48	2	neg	neg	pos	pos	strong
66	70	2	pos	neg	neg	neg	neg
67	48	2	pos	neg	neg	neg	neg
68	55	2	neg	neg	pos	pos	weak
69	61	3	neg	neg	pos	pos	strong
70	48	1	pos	pos	neg	neg	neg
71	66	2	neg	neg	pos	pos	strong
72	52	3	neg	neg	neg	pos	strong
73	47	1	pos	pos	neg	neg	neg
74	68	3	neg	neg	pos	pos	weak
75	56	1	pos	neg	neg	neg	neg